temperature for 24 h. After CH_3OH was added, the mixture was concentrated in vacuo. Chromatography on silica gel with EtOAc/hexane from 40:1 to 2:1 furnished **12** in a yield of 6 g (78%).

29,30-Bis-*O*-{[2-(trimethylsilyl)ethoxy]methyl}nigericinol (13). Compound **12** (6 g, 5.4 mmol) was allowed to reflux with $LiAlH_4$ (1.5 g, 40 mmol) in 100 mL of THF for 3 h under nitrogen. The workup was performed as described for compound **3**; the yield of **13** was 4.4 g (86%).

1-*O*-Isobutyrylnigericinol (14). Compound **13** (4 g, 4.1 mmol) was heated at 40 °C in 100 mL of THF in the presence of tetra-*n*-butylammonium fluoride (800 mg) for 6 h. After filtration and evaporation of the solvent, the residue was chromatographed on silica gel with EtOAc/hexane from 40:1 to 2:1. Compound **14** was isolated in a yield of 2.3 g (72%).

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Registry No. **1**, 28380-24-7; **2**, 127108-23-0; **3**, 138285-64-0; **4**, 124387-94-6; **5**, 132789-24-3; **6**, 132789-25-4; **7**, 138285-65-1; **8**, 124388-03-0; **9**, 138285-66-2; **10**, 138285-67-3; **11**, 138285-68-4; **12**, 138285-69-5; **13**, 138285-70-8; **14**, 138285-71-9.

Perfluorocarbon-Based Antidiabetic Agents[⊥]

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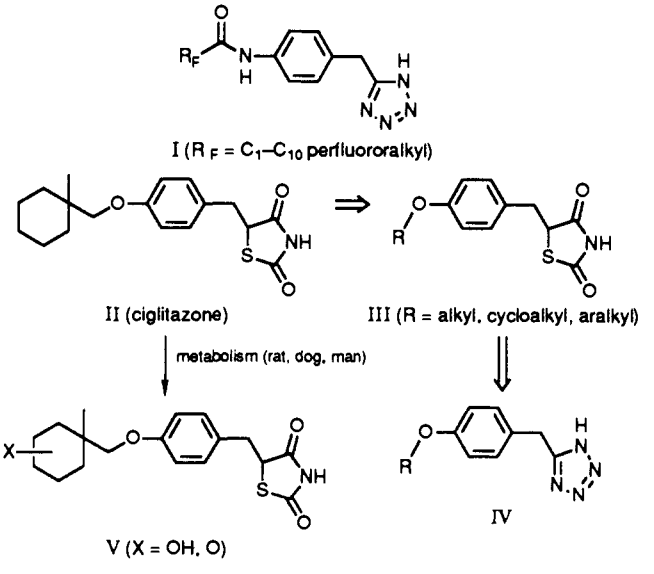
In a preliminary communication (*J. Med. Chem.* **1989**, *32*, 11-13) a series of perfluoro-*N*-[4-(1*H*-tetrazol-5ylmethyl)phenyl]alkanamides (perfluoro anilides I), designed as novel analogues of ciglitazone, were reported to possess oral antidiabetic activity in two genetic animal models of non-insulin-dependent diabetes mellitus (NIDDM): obese (ob/ob) and diabetic (db/db) mice. In this report, the results from a structure–activity relationship (SAR) study of the series I are described. Comprehensive statistical analysis among the 86 analogues screened for blood glucose lowering in ob/ob mice was achieved by a new application of a general statistical procedure which made it possible to make meaningful comparisons between more than 140 separate experiments (N = 2966). Perfluoro anilides I lowered plasma glucose in the hyperglycemic ob/ob and db/db mice but not in euglycemic normal rats. In the hyperinsulinemic ob/ob mouse, decreases in plasma insulin levels paralleled the decline in plasma glucose. Potency and efficacy in the series was shown to be dependent on the length of the perfluorocarbon chain (R_F) of I. Optimal activity occurred with the C_7 and C_8 R_F chains. The more extensive SAR studies reported here, indicated that the lipophilic R_F chain is the most important structural element of I since neither the phenyl nor tetrazole rings present in anilides I were necessary for antihyperglycemic activity while medium length (C_7–C_8) R_F chains, especially the C_7F_{15} chain, were shown to confer antihyperglycemic activity in ob/ob mice to a wide variety of structures.

Recently we reported the synthesis and antidiabetic activity of a series of perfluoro-*N*-[4-(1*H*-tetrazol-5ylmethyl)phenyl]alkanamides[1] (perfluoro anilides, I, Scheme I). These compounds, based on in vivo characterization,[2] appear to have a pharmacologic profile similar to that of ciglitazone[3] (II), the prototype for a new generation of oral antihyperglycemic agents.[4]

In several types of insulin-resistant animals (models of non-insulin-dependent diabetes mellitus (NIDDM)), ciglitazone lowers plasma glucose and improves glucose tolerance by, at least in part, improving insulin responsiveness in peripheral tissues.[5] Ciglitazone does not lower plasma glucose in nondiabetic (normal) or in insulin-deficient animals (models of IDDM). Very large doses of the drug (e.g. > 10 times the amount necessary to lower plasma glucose in insulin resistant animals) do not lower plasma glucose below euglycemic levels, unlike the widely used oral hypoglycemic sulfonylureas.[6]

We began a search for structurally novel antihyperglycemic agents which would possess the pharmacologic profile of ciglitazone but would not contain the antihyperglycemic pharmacophore III[7] (Scheme I). Substitution of a tetrazole nucleus for the acidic thiazolidinedione

Scheme I. Ciglitazone and Analogues



heterocycle generated a lead in this search, but hybrid compounds which combined the benzyltetrazole IV with

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⊥ Dedicated to Professor Joseph F. Bunnett on the occasion of his retirement from the University of California, Santa Cruz.

(1) Kees, K. L.; Cheeseman, R. S.; Prozialeck, D. H.; Steiner, K. E. Perfluoro-*N*-[4-(1*H*-tetrazol-5ylmethyl)phenyl]alkanamides: A New Class of Oral Antidiabetic Agents. *J. Med. Chem.* **1989**, *32*, 11-13.

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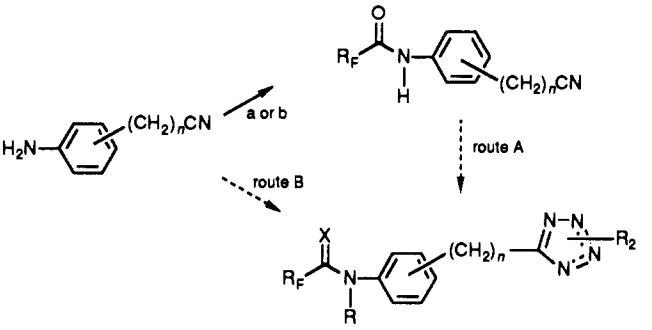
ciglitazone like lipophilic radicals, while active in the genetically obese (ob/ob) mouse, lacked sufficient potency.[8]

A number of the more potent antihyperglycemic thiazolidinediones, including close structural analogues of II, were reported to possess undesirable side effects[7] which could be related to the in vivo metabolism of their lipid fragments. Ciglitazone is known to be metabolized by oxidation of the cyclohexyl ring to hydroxy and oxo derivatives V (Scheme I). All of these known metabolites are active antihyperglycemic agents and several are more potent than the parent II.[9] We thus sought a highly lipophilic entity to append to the benzyltetrazole IV which would resist metabolic oxidation.

Perfluorobutyramide **3** (Wy-49,146; Table I), the first member of the series of perfluoro anilides (I) synthesized, was the first benzyltetrazole we found to have antihyperglycemic potency comparable to ciglitazone in fed ob/ob mice (~40% glucose lowering at 75 mg kg^{-1} day^{-1} × 2). Administration of **3** at very high doses (300 mg kg^{-1} day^{-1} × 4) or over a prolonged period (75 mg kg^{-1} day^{-1} × 9) did not reduce plasma glucose levels in ob/ob mice below the normal glycemic levels of lean mice.[10] Perfluorobutyramide **3** did not decrease plasma glucose levels in normal rats (fasted or fed). However **3**, unlike ciglitazone, was not active in oral glucose tolerance tests administered to ob/ob mice.[2] Subsequently, the perfluorooctanamide **6** (Wy-49,322) was prepared. This compound does improve oral glucose tolerance in ob/ob mice and was shown to be a more potent antihyperglycemic agent than either **3** or ciglitazone,[2] which led to the synthesis of additional analogues in order to further improve potency.

Herein we report the results of our structure–activity relationships (SAR) studies of the antihyperglycemic series of perfluoro anilides I as well as some other pharmacologic effects associated with medium-length perfluorocarbon chains. A new application of a general statistical procedure developed to allow us to make meaningful comparisons between any two compounds or groups of compounds tested in separate experiments is also described.

Scheme II. Representative Synthetic Procedures



compd	route	reagents/conditions[a]
1–16, 19–21	A	*c*
34, 35, 37, 38	A	c
22, 23	A	d, c
24, 25	A	c, e
26, 27	A	c, f
28	B	g, h, e
29	A	c, i
32	B	j, c
33	B	k, c
36	B	c, l, m
39	B	c, l
40–45	A	c, n

[a] (a) R_FCOCl or $(R_FCO)_2O$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, 0 °C; (b) R_FCO_2H, EtOC≡CH, AcOEt, 85 °C, 15 h; (c) NaN_3, NH_4Cl, DMF, 135 °C; (d) $PhCH_2Br$ (excess), K_2CO_3, Me_2CO, reflux; (e) $PhCH_2Br$ (1 equiv), K_2CO_3, Me_2CO, 50 °C; (f) MeI (excess), K_2CO_3, Me_2CO; (g) $C_3F_7C(OH)_2$, pTSA, C_6H_6, reflux, $-H_2O$; (h) $NaBH_4$, THF; (i) LAH (excess), THF, reflux; (j) $CF_3(CH_2)_2CO_2Et$, reflux; (k) $CH_3(CH_2)_5COCO_2H$, DAST, $CFCl_3$, then $4\text{-}H_2NPhCH_2CN$, 0 °C → RT; (l) HCl; (m) C_6F_5COCl, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, 0 °C; (n) NaOH, EtOH.

Chemistry

The perfluoro amides (Tables I–IV) were routinely prepared as shown in Scheme II. Generally the perfluoro acid chloride or anhydride was condensed with an aniline or amine in the presence of triethylamine or diisopropylethylamine. In those cases where the perfluoro acid chloride or anhydride were not commercially available, the acid was coupled directly to the amine with the aid of ethoxyacetylene.[11] Tetrazoles were then formed from the nitriles using an excess of ammonium azide in hot dimethylformamide. This procedure was reliable except in those cases where the perfluoro anilide nitrogen was tertiary (**22** and **23**). In these cases, the perfluoroacyl chain was cleaved to give the primary amide ($R_FCON(R)Ar \rightarrow R_FCONH_2$). The products **22** and **23** were obtained by monitoring (TLC) and quenching the reaction before complete conversion of starting material. Alternatively, the aniline nitrogen was selectively acylated (1 equiv R_FCOCl, 0 °C) in the presence of the tetrazole to give **36**. The tetrazole ring was selectively alkylated (K_2CO_3, benzyl bromide (1 equiv), acetone, 50 °C), which gave an approximately 1:1 mixture of N-1(H) and N-2(H) monoalkylated tetrazole isomers **24** and **25**, easily separable by chromatography. Similarly, dialkylation (K_2CO_3, methyl iodide (xs), acetone, reflux) gave a mixture of tetrazoles

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(6) Goldman, J. M. Oral Hypoglycemic Agents: An Update of Sulfonylureas. *Drugs Today* **1989**, *25*, 689–695.

(7) Sohda, T.; Mizuno, K.; Tawada, H.; Imamiya, E.; Sugiyama, Y.; Fujita, T.; Kawamatsu, Y. Studies on Antidiabetic Agents. I. Synthesis of 5-[4-(2-Methyl-2-phenylpropoxy)benzyl]thiazolidine-2,4-dione(AL-321) and Related Compounds and Studies on Antidiabetic Agents. II. Synthesis of 5-[4-(1-Methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione (ADD-3878) and Its Derivatives. *Chem. Pharm. Bull.* **1982**, *30*, 3563–3573; 3580–3616.

(8) Kees, K. L. Tetrazoles and Their Use As Hypoglycemic Agents. U.S. Patent 4,845,231, 1989.

(9) Sohda, T.; Meguro, K.; Kawamatsu, Y. Studies on Antidiabetic Agents. IV. Synthesis and Activity of the Metabolites of 5-[4-(1-Methylcyclohexylmethoxy)benzyl]2,4-thiazolidinedione (Ciglitazone). *Chem. Pharm. Bull.* **1984**, *32*, 2267–2278 and references therein.

(10) Lean (+/?) mice, $N = 30$, had mean plasma glucose levels = 99 mg/dL (83–115 mg/dL).

(11) Optimum yields were obtained by allowing at least 8 h for the formation of activated perfluoro acid ester, prior to addition of the amine.

26 and 27. Structural assignments for the isomeric tetrazoles were made on the basis of ^1H NMR.[12] Reductive amination of heptafluorobutyraldehyde hydrate ($NaBH_4$) generated perfluoro amine **28**. Amine **29** was obtained by LAH reduction of anilide **6**.[13] The α-gem difluoro derivative **33** was prepared by the action of DAST[14] on 2-oxooctanoic acid followed by treatment (in situ) with 4-aminophenylacetonitrile in the presence of Hunig's base. The derived nitrile was then converted to tetrazole as above. Sodium salts of the tetrazoles were prepared (**40–45, 53,** and **54**) using sodium hydroxide (0.9 equiv) in ethanol.

Biological and Statistical Methods

The biological results (activity column, Tables I–IV) are the mean plasma glucose levels for a particular treatment (a specific drug/dose combination) expressed as a percent of the vehicle group. Obese mice (cells[15] of 7–11 animals each) were administered drug or vehicle (0.5% methyl cellulose) once daily (po) for four consecutive days. Animals were killed by decapitation on the morning of the fifth day, and plasma glucose levels were determined as described.[2]

Since none of these compounds reduced blood glucose levels below the glycemic levels of lean control animals, a biological response floor was imposed. A consequence of this floor was that the more effective compounds, by more uniformly lowering plasma glucose in each animal, produced a relatively small standard deviation per cell compared to less effective compounds, which often showed much greater deviations per cell. This variability from cell to cell violates the basic assumption of homogeneity of variance among different treatment groups and thus created a major obstacle in the comparative analysis of data among experiments from the 86 compounds tested in Tables I–IV. Compounding this problem was a large variation between experiments in the glucose levels of vehicle controls and the modest level of hyperglycemia of the ob/ob mice, which created a narrow response window for the determination of dose–response curves.[16]

Meaningful comparisons between separate experiments were accomplished by using the reciprocal transformation $y = c/x$, where x is the glucose level for each observation in a cell and c was arbitrarily chosen as 160 (the rounded value of the average vehicle means from all the experiments).[16] This reciprocal transformation reduced a 20-fold difference in cell standard deviations to a 6-fold difference in the transformed data (y). To adjust for multiple comparisons, 20 and 75 mg/kg doses of each compound in Tables I–IV were grouped into one of 12 different sets. Each set represented a specified variance of structure. For example, compounds **1–10** comprise a set of compounds that differ only in the length of the perfluorocarbon chain. A two-sided significance level based on a Bonferroni adjustment[17] for multiple comparisons was then calculated for each set of compounds (see Experimental Section). A separate Bonferroni adjustment was made for comparisons of any particular compound to either vehicle control (results in Tables I–IV), ciglitazone or the lead structure **6** (supplementary data[18]).

SAR Results

Hydrocarbon analogues (**32–35**) and polyfluorinated aryl derivatives (**37–38**) of perfluoro anilides **3** and **6** were not active. Pentafluorobenzamide **36** was active in lowering plasma glucose, but did not significantly lower plasma insulin levels.[19] These results indicated that a contiguous chain of perfluorocarbon atoms was necessary for the desired activity. In addition, since anilide **6** had greater potency than **3**, a perfluorocarbon chain length (R_F) dependent activity profile was suggested. Evaluation of the series of perfluoro anilides **1–10** (Table I), which differ only in R_F chain length, did show that efficacy and potency was dependent on the length of the perfluorocarbon chain.[1] Table I summarizes the results obtained at 20 mg kg^{-1} day^{-1} × 4 dosage, which indicate maximum glucose lowering is induced by the C_7–C_{10} perfluorocarbon chains (C_8–C_{11} perfluoroacyl chains, **6–10**). Other members of the series (**1–5**) were active at higher doses, but were not significantly active at 20 mg/kg. Within the C_7–C_{10} group, optimum activity, which included corresponding decreases in plasma insulin levels, occurred with the C_7 and C_8 chains (compounds **6–8**) as described.[1]

Removal of the acidic protons present in perfluoro anilides I by alkylation (compounds **22–27**) modulated activity in both directions relative to **6**. Alkylation of the amide nitrogen marginally improved activity (compare **22** and **23** vs **13** and **8**, respectively). Alkylation of the acidic tetrazole ring lowered activity in the case of **24** vs **6** but otherwise (**25–27**) appeared to have no effect on plasma glucose lowering. This result is in contrast to the ciglitazone series, where alkylation of the acidic thiazolidinedione ring removes antihyperglycemic efficacy.[7] Interestingly however, compounds **25–27** did not produce a parallel decline of plasma insulin levels.[1] These results suggest that the perfluoro anilide induced decreases of plasma glucose and insulin may occur by independent mechanisms.[20,21]

The C_3, C_7, and C_8 R_F chains were used to evaluate the effect of additional structural modifications on activity, with the goal of improving antihyperglycemic potency over the lead anilide **6**. Comparison of positional isomers (ortho, meta, and para) of perfluoro anilides I indicated that R_F chain length was more important than the nature of the

(12) Butler, R. N. Recent Advances in Tetrazole chemistry. In *Adv. Heterocyclic Chem.* Katritzky, A. R., Boulton, A. J., Eds.; Academic Press: New York, 1977; Vol. 21, pp 323–435.

(13) Lewis acid conditions, e.g.: $Bu_4NBH_3CN/BF_3{\cdot}Et_2O$, CH_2Cl_2, reflux, 48 h; AlH_3, THF reflux, 72 h did not reduce **6**.

(14) Hudlicky, M. Fluorination with Diethylaminosulfur Trifluoride and Related Aminofluorosulfuranes. *Org. React* **1988**, *35*, 524–526.

(15) We refer to a group of N animals receiving one dose of one drug/day × 4 as a cell.

(16) The average plasma glucose from controls (N = 294) was 159 mg/dL. Means ranged from 97–257 mg/dL. Standard deviations ranged from 5–100 mg/dL.

(17) Miller, G. M., Jr. *Simultaneous Statistical Inference*, 2nd ed.; Springer-Verlag: New York, 1981; pp 67–70.

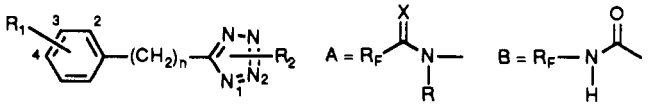
(18) A complete listing of the 12 groups of compounds which constitute Tables I–IV, harmonic and adjusted harmonic means for each compound at 75 and 20 mg/kg doses and the relevant Bonferroni p values are available as supplementary data. log p values (distribution coefficients) for compounds **1–10, 24–28, 35, 40,** and **41** have been obtained by the HPLC method and are available upon request.

(19) Insulin (mean ± SEM, μunit/mL): vehicle control, 208 ± 10; ciglitazone, 138 ± 15 ($p < 0.05$); **36**, 170 ± 15.

(20) Colca et al. have noted that in ciglitazone treated ob/ob mice, circulating insulin levels are decreased prior to glucose lowering: Colca, J. R.; Wyse, B. M.; Sawada, G.; Jodelis, K. S.; Connell, L.; Fletcher-McGruder, B.; Paluzak, B. J.; Diani, A. R. Ciglitazone, A Hypoglycemic Agent: Early Effects on Pancreatic Islets of ob/ob Mice. *Metabolism* **1988**, *37*, 267–280.

(21) Conversely, Stevensen et al. have reported that the glucose lowering effect of englitazone (a ciglitazone analogue) precedes reduction of hyperinsulinemia in ob/ob mice: Stevenson, R. W.; Hutson, N. J.; Krupp, M. N.; Volkmann, R. A.; Holland, G. F.; Eggler, J. F.; Clark, D. A.; McPherson, R. K.; Hall, K. L.; Danbury, B. H.; Gibbs, E. M.; Kreutter, D. K. Actions of Novel Antidiabetic Agent Englitazone in Hyperglycemic Hyperinsulinemic ob/ob Mice. *Diabetes* **1990**, *39*, 1218–1227.

Table I. Effects of Perfluoro Compounds on Plasma Glucose in ob/ob Mice



compd	R_1	R	R_F	X	*n*	R_2	mp, °C	purification method[a] (solvent)[b]	method[c]	*N*	activity[d] dose	activity[d] glucose
1	4A	H	CF_3	O	1	H	203–206	I (ii, v, ix)	1	18	20	104
2	4A	H	C_2F_5	O	1	H	183–185 dec	II (i, iii)	1	29	20	86
3	4A	H	C_3F_7	O	1	H	176–178	III (vii, x)	3	49	20	96
4	4A	H	C_4F_9	O	1	H	173–174	II (ii, iv)	10	28	20	92
5	4A	H	C_6F_{13}	O	1	H	186–188 dec	II (ii, iii)	10	27	20	93
6	4A	H	C_7F_{15}	O	1	H	193–194	II (vii, x)	3	86	20	66*
7	4A	H	C_8HF_{16}	O	1	H	187.5–190.5	II (i, iv)	3	37	20	65*
8	4A	H	C_8F_{17}	O	1	H	195–198 dec	III (vii, x)	10	37	20	63*
9	4A	H	C_9F_{19}	O	1	H	205–209 dec	II (i)	10	28	20	71*
10	4A	H	$C_{10}HF_{20}$	O	1	H	199.5–202	II (i, iv)	10	28	20	62*
11	2A	H	C_3F_7	O	1	H	177–178	I (ii, iii, ix)	3	20	20	91
12	2A	H	C_7F_{15}	O	1	H	175–177	II (ii)	3	10	20	66*
13	3A	H	C_3F_7	O	1	H	148–151	I (ii, v, ix)	3	9	20	86
14	3A	H	C_7F_{15}	O	1	H	163–166	II (ii, iii)	3	29	20	60*
15	4A	H	C_3F_7	O	0	H	266–268	II (xi)	3	11	75	75*
16	4A	H	C_7F_{15}	O	0	H	238–240	II (i)	3	10	20	82
17	4A	H	C_3F_7	O	2	H	161–162	II (vi, iii)	18	9	20	76
18	4A	H	C_7F_{15}	O	2	H	187–189	II (ii)	18	9	20	119
19	4A	H	C_3F_7	O	3	H	143.5–145	II (vi, iii)	19	10	20	72*
20	4A	H	C_7F_{15}	O	3	H	170.5–172.5	II (vi, iii)	19	9	20	63*
21	4A	H	C_7F_{15}	O	4	H	156–158	II (vi, iii)	21	9	20	73
22	3A	$PhCH_2$	C_3F_7	O	1	H	113–116	I (ii, v, ix)	23	40	20	78
23	4A	$PhCH_2$	C_8F_{17}	O	1	H	99–103 dec	II (viii, iii)	23	11	20	55*
24	4A	H	C_7F_{15}	O	1	$PhCH_2$-N_1	160–163	III (i, x)	24	9	20	82
25	4A	H	C_7F_{15}	O	1	$PhCH_2$-N_2	109–113	IV (iv)	25	9	20	68*
26	4A	CH_3	C_7F_{15}	O	1	CH_3-N_1	80–82	I (ii, iii)	26	9	20	67*
27	4A	CH_3	C_7F_{15}	O	1	CH_3-N_2	76–78	I (ii, iii)	26	9	20	70*
28	4A	H	C_3F_7	H_2	1	H	126–128	I (ii, v, ix)	28	11	75	99
29	4A	H	C_7F_{15}	H_2	1	H	138 dec	II (i, iv)	29	10	75	57*
30	4B		$C_3F_7CH_2$		1	H	166.5–168	II (vi, iii)	30	9	20	90
31	3B		$C_3F_7CH_2$		1	H	180–182	II (vi, iii)	30	10	20	93
32	4A	H	$CF_3(CH_2)_2$	O	1	H	173.5–175.5	II (i, iv)	32	29	75	104
33	4A	H	$CH_3(CH_2)_5CF_2$	O	1	H	168–170	III (vii, x)	33	10	75	80
34	4A	H	$CH_3(CH_2)_2$	O	1	H	195–197	II (i, iv)	1	10	75	108
35	4A	H	$CH_3(CH_2)_{16}$	O	1	H	158–161	II (ii)	3	9	20	108
36	4A	H	F_5-Ph	O	1	H	240–242	II (ii)	36	9	20	75*
37	4A	H	3,5-$(CF_3)_2$Ph	O	1	H	228–230	V (ii)	3	10	20	82
38	4A	H	4-CF_3Ph	O	1	H	238–240	II (i)	3	10	75	130
39	4-NH_3Cl				1	H	255–258 dec	II (v, iv)[e]	39	9	75	93
40	4A	H	C_3F_7	O	1	Na^+	233 dec	VI (iv)	40	11	75	67*
41	4A	H	C_7F_{15}	O	1	Na^+	>250	II (xi, iv)	40	10	20	55*
42	2A	H	C_3F_7	O	1	Na^+	146–149	VI (iv)	40	10	20	84
43	3A	H	C_3F_7	O	1	Na^+	155–158	VI (iv)	40	9	20	71*
44	4A	H	C_8F_{17}	O	1	Na^+	>250	VI (ii)	40		NT	
45	4A	H	C_7F_{15}	O	3	Na^+	>220	VI (ii)	40		NT	
ciglitazone										207	20	70*

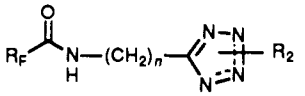
[a] I: HPLC. II: Recrystallization. III: Precipitation. IV: Flash chromatography. V: Material isolated analytically pure from standard aqueous workup. VI: Trituration. [b] Solvents: i = acetone; ii = ethyl acetate; iii = hexanes; iv = ether; v = methanol; vi = THF; vii = DMF, viii = dichloromethane; ix = acetic acid; x = water; xi = ethanol. [c] Synthetic method according to compound number, see Experimental Section. [d] Groups of test animals ($11 \geq N \geq 7$) were administered either drug or vehicle (0.5% methylcellulose) once daily po × 4 days. Results are expressed as the percent plasma glucose concentration of drug treated mice relative to vehicle control animals at the given dose (mg/kg). *Indicates a compound was significantly better than control, $p \leq 0.05/k$ where k is the number of compounds in the set being compared, see Experimental Section. NT: not tested. [e] N: calcd, 33.18; found, 29.32. This material was 99% pure by analytical HPLC.

isomer (compare **3**, **6**, **40**, **41** vs **11–14**, **42**, **43**). Similar results were obtained by removing the perfluoroacyl carbonyl group (by reduction to amines **28** and **29**) and by varying the length of the alkylene linkage between the phenyl and tetrazole rings (n = 0–4; **15–21**: compare n = 1; **3** vs **6** and n = 3; **19** vs **20** (n = 0, 2, 4 decreased activity)). None of these changes, including the "retro" amides **30** and **31**, produced a compound with superior potency to **6**, but the SAR results did suggest that neither the phenyl nor tetrazole rings present in perfluoro anilides I were essential for antihyperglycemic activity. Compounds **46–54** (Table II), in which the phenyl ring was deleted, still showed plasma glucose lowering activity that was dependent, as before, on R_F chain length. Additionally, three of the five compounds lacking the tetrazole moiety **59–64** (Table III) were active in ob/ob mice. These results reinforced our perception that the antihyperglycemic activity observed was a general property of perfluorocarbon chains.

We therefore screened a variety of functional groups containing perfluorocarbon chains in ob/ob mice (Table III, **55–79**; Table IV, **80–91**). Among the perfluorocarbon carboxylic acids tested (**80–84**), perfluorooctanoic (**83**) was unique in its ability to lower glucose levels, but this compound was clearly toxic to ob/ob mice.[22] Simple derivatives of **83**, ester **85**, primary carboxamide **55**, and hy-

(22) Steiner, K. Unpublished results.

Table II. Effects of Perfluoro Compounds on Plasma Glucose in ob/ob Mice



compd	R_F	n	R_2	mp, °C	purification method[a] (solvent)[b]	syn method[c]	N	activity[d] dose	activity[d] glucose
46	C_7F_{15}	0	H	215–218	II (ii)	**39**	10	20	64*
47	C_8F_{17}	2	H	191–193.5	II (vi, iii)	**10**	18	20	65*
48	CF_3	5	H	103–106	II (vi, iii)	**1**	18	20	95
49	C_3F_7	5	H	85.5–87	II (vi, iii)	**3**	9	20	91
50	C_7F_{15}	5	H	122.5–127	II (vi, iii)	**3**	10	20	54*
51	C_8F_{17}	5	H	127–130	II (vi, iii)	**10**	18	20	57*
52	C_8H_{17}	5	H	90–91	II (ii)	**3**	9	20	100
53	C_7F_{15}	5	Na^+	>260	VI (ii)	**40**	9	20	48*
54	C_8F_{17}	5	Na^+	240 dec	VI (ii)	**40**	9	20	62*
ciglitazone							207	20	70*

[a-d] See footnotes, Table I.

droxamic acid **56**, all showed antihyperglycemic activity comparable to the lead **6** in the primary screen. Other active perfluorocarbon derivatives were the alcohols **89** and **91**, carbamate **88**, secondary amide **57**, and carbazide **58**. Five of 12 additional perfluorooctanamides tested (**65**, **67–69**, and **72**) also were found to have significant antihyperglycemic activity in ob/ob mice at 20 mg/kg.

The results of comprehensive statistical analysis of the 86 compounds tested in Tables I–IV support the notion developed during this SAR investigation that the perfluorocarbon chain of anilides I was the most essential element for antihyperglycemic activity. Table V summarizes the most effective antihyperglycemic compounds from Tables I–IV and compares them to the standard ciglitazone and the lead anilide **6**.[18] More potent compounds than ciglitazone contain the C_7 or C_8 R_F chains. Except for the perfluorononanoyl anilide **23**, the compounds in Table V are all perfluorooctanamides. Only three compounds were found to cause a significantly greater decrease of plasma glucose than **6** (**50** and its salt **53**, **56**, and **69**). None of these structures share with **6** the benzyltetrazole fragment, which clearly indicates the importance of the lipophilic C_7F_{15} chain as a causative and potent glucose lowering agent.

Discussion

During the course of these studies, continued pharmacologic profiling of perfluoro anilides **3** and **6** revealed that **6** induced significant liver weight gain and decreased food consumption in various rodents.[2,22] Hepatomegaly and anorexia are known effects of perfluorooctanoic acid (**83**),[23] a substance which could be released from **6** (and all the perfluorooctanamides in Tables I–III) via in vivo hydrolysis. Anilide **3** did not induce these effects in ob/ob mice (20 mg kg^{-1} day^{-1} × 45)[2] even though the analogous release of perfluorobutyric acid would also be expected to produce hepatomegaly.[24] To gain insight into this problem, selected compounds from Tables I, III, and IV were screened for effects on organ weights in normal mice (Swiss CD-1). The results (Table VI) suggest a general liver toxicity associated with the C_7 (and probably C_8) perfluorocarbon chain. Perfluorooctanoic acid **83**, its ethyl ester **85**, and primary carboxamide **55**, the secondary perfluoro anilides **6** and **25**, and tertiary anilides **23** and **26** all induce significant hepatomegaly. Most important, the perfluoroamine **29**, which is not likely to liberate perfluorooctanoic acid in vivo, also causes liver enlargement. Some of these compounds also decreased heart weight but no significant changes in body weight or food intake were observed in this screen (Table VI). Similar adverse effects on organ weights in rodents have been noted with ciglitazone and related analogues[7,25,26].

Unlike thiazolidinediones however, many of the perfluorocarbon compounds in Tables I–IV markedly decreased food intake in ob/ob mice. This additional effect could contribute to plasma glucose lowering in ob/ob mice,[27] but the pattern and magnitude of decreased food consumption induced by the perfluorocarbons varied among experiments and did not appear to correlate with antihyperglycemic efficacy.[28] The apparent lack of a correlation between glucose lowering and the magnitude or pattern of food intake decreases in ob/ob mice, the activity of **6** in the oral glucose tolerance test (which was preceded by an overnight fast)[2,29] and the activity of **3**, **6**, and **7** in the diabetic db/db mouse in which decreases in food consumption did not occur, suggest that mechanisms other than food restriction contribute to the pharmacologic profile observed.

Perfluoro anilide **6**, by design of structure and indicated by its sustained antihyperglycemic action after withdraw-

(23) Olson, C. T.; Anderson, M. E. The Acute Toxicity of Perfluorooctanoic and Perfluorodecanoic Acids in Male Rats and Effects on Tissue Fatty Acids. *Toxicol. Appl. Pharmacol.* **1983**, *70*, 362–372.

(24) Just, W. W.; Gorgas, K.; Hartl, F.-U.; Heinemann, P.; Salzer, M.; Schimassek, H. Biochemical Effects and Zonal Heterogeneity of Peroxisome Proliferation Induced by Perfluorocarboxylic Acids in Rat Liver. *Hepatology* **1989**, *9*, 570–581.

(25) Meguro, K.; Fujita, T. Thiazolidinedione Derivatives, Useful as Antidiabetic Agents. U.S. Patent 4,687,777, 1987.

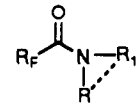
(26) Two thiazolidinedione analogues of ciglitazone currently in clinical trials apparently do not adversely affect organ weights. For reviews and commentary on CS-045 and pioglitazone, see: Kees, K. L. *Chemtracts Org. Chem.* **1990**, *3*, 314–319; **1991**, *4*, 82–86.

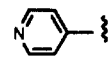
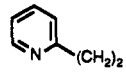
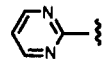
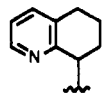
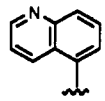
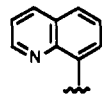
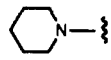
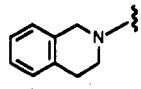
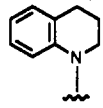
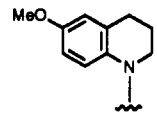
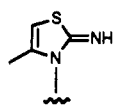
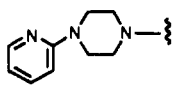
(27) Lavine, R. L.; Voyles, N.; Perino, P. V.; Recant, L. The Effect of Fasting on Tissue Cyclic AMP and Plasma Glucagon in the Obese Hyperglycemic Mouse. *Endocrinology* **1975**, *97*, 615–620.

(28) Food intake decreases ranged from negligible to 50% over the 5-day experiments. The rate of these changes varied from a steady, gradual decline over the 5 days to a steep drop on days 4 and 5. The most extreme example of this is perfluorononanoic acid (**84**). Food consumption by ob/ob mice was negligable after 3 days of **84** administered 75 mg kg^{-1} day^{-1}. In general no significant body weight changes occurred. Exceptions were **83** and **84** (weight loss) and **4** and **5** (weight gain).

(29) Fasting exacerbates glucose intolerance in the oral glucose tolerance test administered to ob/ob mice: Genuth, S. M.; Przybylski, R. J.; Rosenberg, D. M. Insulin Resistance in Genetically Obese, Hyperglycemic Mice. *Endocrinology* **1971**, *88*, 1230–1238.

Table III. Effects of Perfluoro Compounds on Plasma Glucose in ob/ob Mice



compd	R_1	R	R_F	mp, °C	purification[a] (solvent)[b]	syn method[c]	N	activity[d] dose	activity[d] glucose
55	H	H	C_7F_{15}			*e*	19	20	65*
56	H	OH	C_7F_{15}	122–124	I (ii, v, ix)	**3**	10	20	52*
57	H	$CH_3(CH_2)_3$	C_7F_{15}	38–40	II (iii)	**3**	9	75	70*
58	H	$NHCOC_7H_{15}$	C_7F_{15}	135–137	II (ii)	**3**	10	75	74*
59	H	$PhCH_2$	C_7F_{15}	88–90	II (iii)	**3**	10	20	106
60	$CH_3(CH_2)_3$	$PhCH_2$	C_7F_{15}	*f*	V	**3**	10	75	61*
61	H	Ph	C_7F_{15}	105–108	II (iii, iv)	**3**	10	20	74*
62	H	4-FC_6H_4	C_7F_{15}	109–111	II (iii)	**3**	10	20	91
63	H	4-MeC_6H_4	C_7F_{15}	98–100	II (iii, iv)	**3**	10	75	58*
64	H	4-$PhCH_2CN$	C_6F_{15}	170–173	III (ii)	**10**	10	75	69*
65	H		C_7F_{15}	38–40	I (ii, iii)	**3**	9	20	56*
66	H		C_7F_{15}	140–141	II (v)	**3**	10	20	74
67	H		C_7F_{15}	114–116	I (ii, iii)	**3**	11	20	66*
68	H		C_7F_{15}	58–60	II (ii)	**3**	10	20	63*
69	H		C_7F_{15}	130–132	II (viii)	**3**	11	20	49*
70	H		C_7F_{15}	136–138	II (ii, iii)	**3**	9	20	85
71	H		C_7F_{15}	92–95	II (v)	**3**		NT	
72	H		C_7F_{15}	142–145	II (ii)	**3**	11	20	64*
73	H		C_7F_{15}	57–59	II (v)	**3**		NT	
74			C_7F_{15}	*f*	IV (ii, iii)	**3**		NT	
75			C_7F_{15}	41–43	I (ii, iii)	**3**	11	20	87
76			C_7F_{15}	151–153	II (v)	**3**	10	20	115
77			C_7F_{15}	55–58	II (v)	**3**	10	20	132
78			C_7F_{15}	165–168	VI (iii)	**3**	9	20	94
79			C_7F_{15}	79–81	II (ii)	**3**	10	20	76
ciglitazone							207	20	70*

[a–d,*] See footnotes, Table I. [e] Purchased from PCR. [f] Oil.

Table IV. Effects of Perfluoro Compounds on Plasma Glucose in ob/ob Mice

R_F—X

compd	R_F	X	mp, °C	purification method[a] (solvent)[b]	syn method[c]	N	activity[d] dose	activity[d] glucose
80	C_3F_7	CO_2H			*e*	9	20	101
81	C_4F_9	CO_2H			*f*	9	75	133*
82	C_6F_{13}	CO_2H			*g*	20	75	107
83	C_7F_{15}	CO_2H			*h*	19	20	60*
84	C_8F_{17}	CO_2H			*g*	20	75	124
85	C_7F_{15}	CO_2Et			*h*	8	20	72*
86	C_8F_{17}	SO_2NHEt			*h*	9	20	90
87	$C_7F_{15}CH_2$	NH_2			*h*	9	20	84
88	$C_7F_{15}CH_2$	$NHCOOCH_3$	65–68	I (ii, iii)	**3**	10	75	57*
89	$C_7HF_{14}CH_2$	OH			*g*	10	20	67*
90	C_7F_{15}	H		*i*	*h*	10	75	90
91	C_8F_{17}	PhCHOH	70–72	VI (viii)	*j*	10	75	64*
ciglitazone						207	20	70*

[a-d,*] See footnotes, Table I. [e] Purchased from Aldrich. [f] Purchased from Columbia. [g] Purchased from Riedel-de Haen. [h] Purchased from PCR. [i] This material was too volatile to obtain satisfactory combustion analysis. [j] Prepared by the method of Gassman and O'Reilly (*J. Org. Chem.* **1987**, *52*, 2481).

Table V. Comparison of Most Effective Compounds from Tables I–IV vs Ciglitazone and 6

compd[a]	N	% of ciglitazone[b]	% of 6[c]
6	86	95	–
14	29	86*	90
23	11	80*	84
41	10	79*	84
50	10	77*	82*
51	18	82*	87
53	9	68*	72*
56	10	75*	79*
69	11	70*	74*

[a] All compounds compared at 20 mg/kg dose × 4 days. Vehicle harmonic mean (N = 294) = 139 mg/dL. [b] Adjusted harmonic mean (N = 207) = 97 mg/dL. [c] Adjusted harmonic mean = 92 mg/dL. *$p \leq 0.0026$.

Table VI. Effects of Perfluoro Compounds on Liver, Heart, and Body Weights in Normal Mice[a]

compd	body wt, g[b] pre	body wt, g[b] post	heart wt. (mg/10 g b.w.)	liver wt. (g/10 g b.w.)
6	30.5 ± 0.3	31.4 ± 0.4	39 ± 1	1.255 ± 0.036*
23	29.8 ± 0.4	31.3 ± 0.8	39 ± 1*	1.114 ± 0.027*
25	30.3 ± 0.5	31.4 ± 0.4	41 ± 1	0.787 ± 0.021*
26	29.9 ± 0.4	31.1 ± 1.0	41 ± 1	0.942 ± 0.022*
29	30.3 ± 0.6	31.2 ± 0.9	39 ± 1*	0.856 ± 0.030*
33	30.4 ± 0.6	31.7 ± 0.9	43 ± 1	0.671 ± 0.013
39	29.8 ± 1.2	30.1 ± 1.6	43 ± 2	0.591 ± 0.025
58	30.6 ± 0.8	30.6 ± 0.9	37 ± 1*	1.440 ± 0.027*
60	30.8 ± 0.5	31.1 ± 1.5	40 ± 1	1.182 ± 0.021*
67	30.7 ± 0.9	32.4 ± 1.1	39 ± 1*	1.085 ± 0.022*
vehicle[c]	30.9 ± 0.7	31.1 ± 0.7	44 ± 1	0.637 ± 0.015

[a] Swiss CD-1 mice (Charles River), in groups of six were dosed orally once per day for 4 days with 50 mg/kg of drug or vehicle (2% Tween 80/saline). [b] Figures represent mean body weights on day 1 and day 5, respectively. [c] Vehicle-treated animals were housed in three cages, three mice per cage. *$p < 0.05$ using Bonferroni's t test.

ing its administration to diabetic db/db mice,[2] can be classified as a nonmetabolizable lipophilic acid. Consistent with other members of this broad classification,[30] anilide **6** significantly lowers plasma lipids (triglyceride and cholesterol) in normal fed rats,[2] induces hepatomegaly in rodents, most likely via peroxisomal enzyme proliferation, and possesses antihyperglycemic effects in rodent models of NIDDM. From the limited data available we cannot determine to what extent, if any, each of the various pharmacologic actions contribute to glucose lowering in the ob/ob mouse.

Summary and Conclusions

We began this work with the objective of discovering a structurally novel antihyperglycemic agent which would possess a ciglitazone pharmacologic profile. Our interest in generating nonoxidizable-lipid-containing acidic azoles led to the synthesis of a series of perfluoro anilides I. These compounds, studied in two genetic animal models of NIDDM and in normal rats, do mirror some of the actions of ciglitazone and related thiazolidinediones. However, a significant difference was found in the SAR of the two series. Whereas the thiazolidinediones require the acidic proton on the heterocycle for activity, perfluoro anilides I do not. Removal of the acidic tetrazole hydrogen in I by alkylation produced isomeric N-1(H) and N-2(H) alkyl tetrazoles that retained antihyperglycemic efficacy. Unlike their N-1(2)H parents, these alkylated materials did not induce a parallel decline in plasma insulin levels, indicating plasma glucose and insulin levels may be decreased by separate mechanisms.

Further SAR studies demonstrated that neither the tetrazole nor phenyl rings present in perfluoro anilides I were necessary for antihyperglycemic activity. Rather, it is the lipophilic perfluorocarbon chain of I that is the most important structural element. Perfluorocarbon chains, in particular the C_7F_{15} chain, confer antihyperglycemic activity to a wide variety of structures. However, tests in normal mice suggest the C_7F_{15} chain is a general liver toxin. A persistent anorexic effect induced by medium-length perfluorocarbon chains was also observed. Either of these effects, as well as other more direct effects on insulin action, could contribute to the plasma glucose lowering in ob/ob mice. However, the relative importance of these factors could not be determined from the data available.

Although the undesirable effects of perfluorocarbon-based antidiabetic agents described here prohibit our future study of these compounds, they may prove to be useful probes for the unraveling of the mechanisms involved in deranged carbohydrate and lipid metabolism in models of NIDDM. In addition, our solution to the statistical analysis problem presented by the need to make meaningful comparisons between more than 140 separate experiments (N = 2966) may help others in this and allied fields.

(30) Bar-Tana, J.; Ben-Shoshan, S.; Blum, J.; Migron, Y.; Hertz, R.; Pill, J.; Rose-Khan, G.; Witte, E. C. Synthesis and Hypolipidemic and Antidiabetogenic Activities of β,β,β′,β′ Tetrasubstituted, Long Chain Dioic Acids. *J. Med. Chem.* **1989**, *32*, 2072–2080.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with assigned structures. NMR spectra were recorded on a Varian XL-300 at 300 MHz, a Varian XL-100 at 100 MHz or a Varian FT-80A at 80 MHz. Mass spectra were recorded on a Kratos MS-25 or MS-50. IR spectra were recorded with a Perkin-Elmer 299 infrared spectrophotometer. Elemental analyses were recorded with a Perkin-Elmer 240C elemental analyzer, and all compounds were within 0.4% of theoretical value unless otherwise noted.

Representative Synthetic Procedures (Scheme II). 2,2,3,3,4,4,4-Heptafluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]butanamide (3). A mixture of 4-aminobenzyl cyanide (9.2 g, 70 mmol, Aldrich), diisopropylethylamine (10.8 g, 84 mmol), and CH_2Cl_2 (350 mL) was cooled in an ice bath under a nitrogen atmosphere. To this mixture was added dropwise perfluorobutyryl chloride (neat, 10.4 mL, 70 mmol, Aldrich). The reaction mixture was allowed to warm gradually to room temperature and was stirred overnight at ambient temperature (15 h). The mixture was then cooled in ice and stirred with 10% HCl solution. The solid was filtered off and washed with water. After air drying, the product was further dried in a vacuum for several hours. The 2,2,3,3,4,4,4-heptafluoro-*N*-[(4-cyanomethyl)phenyl]butanamide so obtained (17.5 g, 76%) was sufficiently pure for further use (homogeneous by thin layer chromatography, 70:30 hexane–ethyl acetate). A sample recrystallized from CH_2Cl_2 yielded silvery plates, mp 116–118 °C.

A mixture of 2,2,3,3,4,4,4-heptafluoro-*N*-[4-(cyanomethyl)-phenyl]butanamide (17.5 g, 53 mmol), sodium azide (17.3 g, 0.27 mol), and ammonium chloride (14.1 g, 0.27 mol) was heated in DMF (250 mL) at 130–135 °C (oil bath) under a nitrogen atmosphere for 20 h. Enough water was then added to the hot reaction mixture to dissolve all suspended salts, and the mixture was allowed to cool to room temperature. The reaction vessel was cooled in ice, and additional water was then added to produce a white precipitate which was filtered, washed with water, and dried under vacuum. The white solid product, **3** (8.7 g, 44%, mp 176–179 °C), was analytically pure as isolated. A sample recrystallized from acetone (minimum amount)/diethyl ether melted at 176–178 °C.

2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11-Eicosafluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]undecanamide (10). To a solution of 11-hydroperfluoroundecanoic acid (10 g, 18 mmol, PCR) in ethyl acetate (100 mL) was added ethoxyacetylene (6.4 g, 92 mmol), and the mixture was refluxed 15 h under nitrogen atmosphere. The mixture was cooled to room temperature and 4-aminobenzyl cyanide (3.1 g, 24 mmol) was added in one portion. Stirring at room temperature was continued for 24 h. The precipitate (1.5 g) was collected on a Buchner funnel and air dried. The filtrate was washed successively with several portions of 10% HCl and saturated aqueous NaCl solutions, dried over $MgSO_4$, filtered, and concentrated at the rotary evaporator. The residue was dissolved in a minimum amount of ethyl acetate on the steam bath, diluted with hexane, and cooled to room temperature. The resulting brown solid was filtered, washed with hexane and dried under vacuum to give an additional 3.6 g of product, mp 176–178 °C, identical with the precipitate first obtained.

A mixture of 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11-eicosafluoro-*N*-[4-(cyanomethyl)phenyl]undecanamide (5 g, 7.6 mmol), sodiu azide (2.5 g, 38 mmol), ammonium chloride (2 g, 38 mmol), and DMF (70 mL) were heated in a 135 °C oil bath for 18 h. Enough water was added to the hot reaction mixture to dissolve all of the solids and the mixture was allowed to cool to room temperature. When a precipitate began to form more water was added with vigorous stirring. The precipitate was collected by filtration as a dark brown paste. This material was dissolved in warm acetone (steam bath), diluted with ether, and stored at 0 °C. The solution was decanted and the solid triturated with ether. The product was then collected by filtration and air dried to give 2.7 g (51% yield) of **10** as a tan solid.

2,2,2-Trifluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]-acetamide (1). A mixture of 4-aminobenzyl cyanide (20 g, 152 mmol), diisopropylethylamine (19.6 g, 152 mmol), and CH_2Cl_2 (400 mL) was cooled in ice under a nitrogen atmosphere. Trifluoroacetic anhydride (31.8 g, 152 mmol) in CH_2Cl_2 (50 mL) was added dropwise, and the reaction was allowed to warm gradually to ambient temperature overnight. The reaction mixture was diluted with ethyl acetate and washed successively with 5% HCl and saturated brine solutions, dried over $MgSO_4$, filtered, and concentrated with a rotary evaporator to give a tan solid (38.68 g), which was recrystallized from ethyl acetate/hexane mixture to give 27 g (combined two crops, 77% yield) of 2,2,2-trifluoro-*N*-[4-cyanomethyl)phenyl]acetamide as tan crystals, mp 148–150 °C.

A mixture of 2,2,2-trifluoro-*N*-[4-(cyanomethyl)phenyl]acetamide (7.49 g, 33 mmol) sodium azide (10.67 g, 164 mmol), ammonium chloride (8.78 g, 164 mmol), and DMF (200 mL) were heated in a 130 °C oil bath under a nitrogen atmosphere overnight. Water was added until all suspended salts were dissolved, and the mixture was partitioned between saturated aqueous brine solution and ethyl acetate. The extracts were dried over $MgSO_4$, filtered, and concentrated with a rotary evaporator to give 12.2 g of a tan oil. The product was purified by HPLC, yielding the title compound as a white solid.

2,2,3,3,4,4,5,5,6,6,7,7,7-Pentadecafluoro-*N*-[4-(1- and 2-benzyltetrazol-5-ylmethyl)phenyl]octanamide (24 and 25). A mixture of 2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]octanamide (**6**, 5 g, 8.76 mmol), benzyl bromide (1.5 g, 8.77 mmol), and anhydrous potassium carbonate (2.89 g, 20.94 mmol, pulverized with mortar and pestle) were heated in acetone (50 mL) at 50 °C for 15 h. Water was then added to the hot mixture until all of the salts were dissolved. Upon cooling to room temperature a white precipitate formed. Additional water was added, and the mixture was cooled in ice. The precipitate was collected on a Buchner funnel and dried for several hours under reduced pressure (abderhalden apparatus, EtOH, ↑↓) to give 1.2 g of the polar isomer (N-1(H)-alkylated tetrazole), mp 160–163 °C. The filtrate was diluted with EtOAC and washed with saturated brine solution, dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator. The residue was chromatographed on a short silica gel column (elution with ether) to give 1.5 g of the nonpolar isomer (N-2(H)-alkylated tetrazole).

Note: The isomer assignments were derived from ^1H NMR:[12] **24** (80 MHz, DMSO-d_6) δ 5.68 (s, CH_2Ph); **25** (80 MHz, DMSO-d_6) δ 5.85 (s, CH_2Ph); **26** (300 MHz, $CDCl_3$) δ 3.9 (s, CH_3); **27** (300 MHz, $CDCl_3$) δ 4.36 (s, CH_3).

2,2,3,3,4,4,4-Heptafluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)-phenyl]butanamide, Sodium Salt (40). Tetrazole **3** (29 g, 78 mmol) was dissolved in 400 mL of absolute ethanol. Sodium hydroxide (70 mmol) was added, and the mixture was stirred at room temperature for several hours after all of the hydroxide had dissolved. Activated charcoal was added to the dark brown solution, and the mixture was stirred 2 h at room temperature, then filtered through a short pad of Celite, and concentrated on the rotary evaporator. The residue was stirred overnight with 1 L of ether–hexane (3:1) mixture to remove excess tetrazole. The salt was collected on a Buchner funnel and allowed to air dry. The hydrated material was partially dried using a rotary evaporator (vacuum pump) and 75 °C water bath for 6 h to yield 28 g of the title compound.

Other salts listed in Table I were similarly prepared except that ethyl acetate was used in the trituration step when the perfluorocarbon chain length was greater than C_3F_7.

2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-Heptadecafluoro-*N*-(phenylmethyl)-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]nonanamide (23). A mixture of 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-*N*-[4-cyanomethyl)phenyl]nonanamide (3 g, 6.92 mmol, prepared as in **10** above), benzyl bromide (1.77 g, 10.38 mmol), and potassium carbonate (1.43 g, powdered) were refluxed in acetone (50 mL) for 18 h. Another 1.8 g of benzyl bromide was added and reflux continued an additional 24 h. The reaction mixture was cooled to room temperature, water was added, and the mixture was extracted with ethyl acetate. The extracts were combined, washed with saturated brine solution, dried over $MgSO_4$, filtered and concentrated. The residual dark amber oil was purified by HPLC to give 3 g of desired tertiary amide as a yellow solid.

A mixture of the perfluoro-*N*-(phenylmethyl)-*N*-[4-(cyanomethyl)phenyl]nonanamide (2 g, 3 mmol), sodium azide (0.91 g, 14 mmol), and ammonium chloride (0.75 g, 14 mmol) were heated

in DMF (15 mL) at 135 °C for 7 h. The reaction mixture was cooled to room temperature. Water was added until homogeneity was achieved and the mixture was partitioned between saturated brine solution and ethyl acetate. The organic phase was washed with saturated brine solution, dried over $MgSO_4$, filtered, and concentrated on the rotary evaporator to give an orange oil. The oil hardened to a solid upon standing at room temperature overnight. The solid was recrystallized from CH_2Cl_2/hexane mixture to give the title compound **23** as a tan solid.

2,2,3,3,4,4,4-Heptafluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]butanamine (28). Heptafluorobutanal hydrate (30 g, 139 mmol), 4-aminobenzyl cyanide (18.4 g, 139 mmol), and pTSA (catalytic amount) were refluxed in benzene (300 mL) using a Dean-Stark trap to collect the water liberated. When the expected amount of water had been collected, the solvent was removed from the reaction mixture using a rotary evaporator and was replaced by THF (300 mL). This mixture was cooled in ice under a nitrogen atmosphere and treated with sodium borohydride (5.36 g, 139 mmol). The reaction was kept at 0 °C for 2 h, then allowed to warm up to ambient temperature gradually and stirred overnight. The mixture was then cooled in ice, 5% HCl solution was added cautiously to quench excess borohydride, and the resulting mixture was partitioned between saturated brine solution and ethyl acetate. The organic phase was dried over $MgSO_4$, filtered, and concentrated. The residue (41.6 g) was purified by HPLC to give 10 g of the desired heptafluorobutylaniline as a yellow oil.

A mixture of 2,2,3,3,4,4,4-heptafluoro-*N*-[4-(cyanomethyl)phenyl]butanamine (5 g, 16 mmol), sodium azide (5.2 g, 80 mmol), and ammonium chloride (4.28 g, 80 mmol) were heated in DMF (100 mL) at 135 °C for 18 h. The mixture was cooled and concentrated on the rotary evaporator (vacuum pump, 50 °C bath temperature). The residue was triturated with ethyl acetate and filtered. The filtrate was concentrated to give 8.4 g of crude product. HPLC purification gave 4.37 g of the title compound **28** as an off-white solid.

2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-Pentadecafluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]octanamine (29). Perfluorooctanamide **6** (4.5 g, 7.9 mmol), lithium aluminum hydride (0.6 g, 15.8 mmol), and THF (40 mL) were refluxed for 7 days under nitrogen atmosphere. The reaction mixture was cooled in an ice bath and treated sequentially with 15% aqueous NaOH solution (5 mL), water (20 mL), and 10% aqueous HCl solution (until pH = 3). The mixture was extracted with several portions of ethyl acetate. The combined extracts were washed with saturated brine solution, dried over $MgSO_4$, filtered, and concentrated on the rotary evaporator. The crude product was recrystallized from hot acetone (minimum amount to achieve homogeneous solution) ether mixture at 0 °C. The product was collected and dried under vacuum to yield 1.5 g of the title compound as a brown solid.

2,2-Difluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]octanamide (33). A mixture of 2-oxooctanoic acid (5 g, 31.6 mmol, Sigma) and fluorotrichloromethane (freon 11, 150 mL) were cooled in an ice bath under nitrogen atmosphere. Diethylamidosulfur trifluoride (DAST, 9.2 g, 56.9 mmol) was added in one portion (neat) and the reaction allowed to warm up gradually to ambient temperature overnight. The mixture was recooled in ice and treated with a mixture of diisopropylethylamine (6.6 mL) and (4-aminophenyl)acetonitrile (5 g, 37.9 mmol) in dichloromethane (25 mL). The resulting mixture was kept at 0 °C for several hours, then allowed to warm to ambient temperature. Stirring was continued for 15 h at room temperature. The reaction was then cooled in ice and quenched with saturated aqueous $NaHCO_3$ solution. The mixture was extracted with several portions of dichloromethane. The extracts were washed with saturated aqueous brine solution, dried over $MgSO_4$, and concentrated. The residue was purified by HPLC to give 2.5 g of desired 2,2-difluorooctananilide (27% based on starting 2-oxooctanoic acid).

A mixture of 2,2-difluoro-*N*-[4-(cyanomethyl)phenyl]octanamide (2.37 g, 8.06 mmol), sodium azide (2.62 g, 40.3 mmol), and ammonium chloride (2.14 g, 40.3 mmol) in DMF (40 mL) were heated in a 135 °C oil bath for 15 h. Enough water was added to the hot reaction mixture to dissolve all suspended solids and the solution was allowed to cool. Upon cooling to room temperature a precipitate formed. The mixture was cooled in ice and additional water was added. The product was collected, washed with water and ether, and dried under vacuum (abderhalden apparatus) to give 1.2 g of **33** as a tan solid.

4-(1*H*-Tetrazol-5-ylmethyl)aniline Hydrochloride (39). Sodium azide (24.59 g, 378.3 mmol), ammonium chloride (20.24 g, 378.3 mmol), and 4-aminobenzyl cyanide (10 g, 75.65 mmol) were heated in DMF (200 mL) at 130 °C for 48 h. The mixture was concentrated on the rotary evaporator (using a conventional vacuum pump and a 80 °C water bath). The residue was partitioned between ethyl acetate and saturated aqueous NaCl solution. The organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by HPLC to give 12 g of tan oil. A portion of this material was dissolved in methanol and treated with anhydrous HCl. The solvent was removed, and the resulting solid was triturated with ether, filtered and dried under vacuum, to give **39** as a tan solid. The product did not produce satisfactory combustion analysis (nitrogen 3.77% low) but gave satisfactory NMR, IR, and mass spectral data. The sample was 99% pure by analytical HPLC.

2,3,4,5,6-Pentafluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]benzamide (36). A mixture of diisopropylethylamine (7.78 mL, 44.6 mmol) and 4-(1*H*-tetrazol-5ylmethyl)aniline hydrochloride (4.5 g, 21.3 mmol) in dichloromethane (250 mL) was cooled in an ice bath under nitrogen atmosphere. Pentafluorobenzoyl chloride (3.1 mL, 21.3 mmol) in dichloromethane (50 mL) was added dropwise. The mixture was held at 0 °C for several hours, then allowed to warm up gradually, and stirred overnight at room temperature. The precipitate was collected and partitioned between ethyl acetate and 10% aqueous HCl solution. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The residue was recrystallized from ethyl acetate to give 1.1 g of the title compound as a white solid. The filtrate from above produced second and third crops of material which gave an additional 2 g of product.

Representative Antidiabetic Screening Procedure. Female obese (ob/ob) mice obtained from Jackson Laboratories (Bar Harbor, ME) were used 10 weeks after birth. Drug or vehicle (0.5% methyl cellulose) was administered once each day for the number of days indicated. Animals were fed ad libitum. On the morning following the last day of dosing, the mice were decapitated and a blood sample of 0.1 mL was obtained and added to 0.2 mL normal saline containing 1200 units of Trasylol and 2.4 mg EDTA. Plasma glucose was determined on an Abbott VP autoanalyzer.

Organ and Body Weight Study in Normal Mice. Sixty-nine Swiss CD-1 mice (Charles River, 20–25 g/each) were divided into 10 groups of six (for each test compound in Table III). Control mice were divided into three groups of three. Mice were weighed and then administered with test compound (50 mg/kg) or vehicle (2% Tween 80/saline) by gavage each afternoon for four consecutive days. On the morning of the fifth day animals were weighed, killed (halothane), and organ weights determined.

Statistical Methods. The data were analyzed with an incomplete unbalanced block design consisting of 30 blocks (experiments) and 141 different drug/dose combinations (treatments) with approximately 8–10 observations in each cell. The vehicle control was the only treatment to appear in every block. Various doses of the standard reference compound ciglitazone and the lead compound **6** appear in many of the experiments, but not all. Several compounds appear in only one experiment.

Standard deviations of the original data ranged from 5 to 100 mg/dL. By using the regression of the logarithmic value of the cell standard deviations against the logarithmic value of the cell means, the reciprocal transformation ($y = c/x$) was chosen to reduce the differences among the cell standard deviations.[31]

Although the transformed data were analyzed, the results were transformed back to the original units for presentation purposes. As a result, the mean values presented in Table I and in the supplementary data are actually the harmonic means of the original data.

An adjusted (harmonic) mean response was obtained for each drug/dose combination using the least squares means from the SAS(#) General Linear Model procedure (PROC GLM). The magnitude of adjustment to each treatment mean was the dif-

(31) Box, G. E. P.; Hunter, W. G.; Hunter, J. S. *Statistics for Experimenters*; John Wiley & Sons, Inc: New York, 1978; pp 231–238.

ference between the overall vehicle response and the mean response for the experiments containing the particular treatment. For example, for those treatments which appear only in experiments with lower than average responses, the adjusted harmonic mean is higher than the corresponding mean cell response; for those treatments which appear only in experiments with higher than average responses, the adjusted mean is lower than the corresponding cell means.

Each drug/dose combination was compared to the vehicle control response, the response for the appropriate dose of the standard reference compound, ciglitazone, and the lead compound **6**. To adjust for multiple comparisons, the 20 and 75 mg/kg doses of each compound were grouped into one of the 12 different sets (see text). A two-sided significance level ($p < 0.05$) based on a Bonferroni adjustment[17] of multiple comparisons was calculated for each set of compounds by dividing 0.05 (nominal significance level) by the number of comparisons being made within the set. The Bonferroni adjustment was made separately for the comparisons to vehicle control (results in Tables I–IV) ciglitazone and **6** (see supplementary data). A comparison was declared significant (indicated by *) if the observed p value was less than the Bonferroni-adjusted p value for that group of compounds relative to vehicle control.

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Supplementary Material Available: A complete listing of the 12 groups of compounds which constitute Tables I–IV, harmonic and adjusted harmonic means for each compound at 75 and 20 mg/kg doses, and the relevant Bonferroni p values (14 pages). Ordering information is given on any current masthead page.

Synthesis and Antibacterial Activity of Some Novel 6-Methyl- and 6-Propenyl-Substituted Carbapenems

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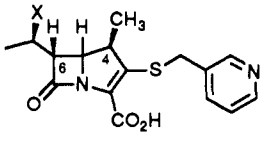
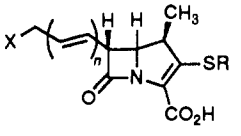
The synthesis and antibacterial activity of a number of 6-methyl- and 6-propenyl-substituted carbapenems is described. The 6-(hydroxymethyl)- and 6-(aminomethyl)carbapenems possessed more potent antibacterial activity in vitro than their respective 6-(1′(*R*)-hydroxyethyl) or 6-(1′(*R*)-aminoethyl) counterparts. However, because of reduced stability, the 6-(aminomethyl)carbapenem was found to be inactive in vivo. All 6-hydroxypropenyl or 6-aminopropenyl derivatives that were prepared were less active than their respective 6-heteroethyl-substituted analogues.

Introduction

Replacement of the hydroxyl function in the 6-(1′-hydroxyethyl) side chain of carbapenems of the thienamycin class (e.g. **1**), with an amino group results in compounds (e.g. **2**[1]) which possess improved activity against Gram-negative bacteria, notably *Pseudomonas aeruginosa*. A part of the work undertaken to elucidate the structure–activity relationship of this group of compounds involved efforts to prepare a 6-aminomethyl derivative (**4**, $n = 0$)[2] and its vinylogue, the 6-(3′-aminoprop-1′-enyl) derivative (**4**, $n = 1$). This work, along with the synthesis and antibacterial activity of some novel 6-(hydroxypropenyl) derivatives [e.g. **3** ($n = 1$)] is described.



1: X = OH
2: X = NH_2

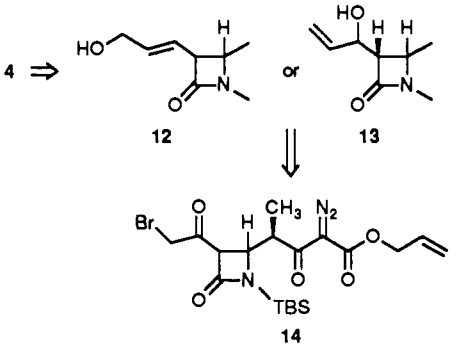


3: X = OH
4: X = NH_2

Chemistry

The preparation of the 6-(hydroxymethyl)carbapenem (**5**) has already been reported.[3] It was felt that this compound could be converted to the 6-aminomethyl analogue (**7**) using methodology first described by Bachi[4] and developed for use in the synthesis of the present series of compounds by Banville.[1] This involved the Mitsunobu[5,6] reaction of the alcohol **5** with hydrazoic acid to give the azide **9**. Reaction of the azide with triphenylphosphine

Scheme I[a]



[a] TBS = *tert*-butyldimethylsilyl.

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‡ Wallingford, CT location.

(1) Banville, J.; Rémillard, R.; Fung-Tomc, J.; Desiderio, J.; Michel, A.; Ménard, M.; Kessler, R.; Partyka, R. 6-(1-Aminoalkyl)-1β-methyl carbapenems: synthesis and in vitro and in vivo activities. 30th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, GA, Oct 21–24, 1990; Abstract 902. A manuscript describing this work is in preparation. 6-(Aminoethyl)carbapenems have been previously described. However, they lacked a 4β-methyl substituent and were described as being extremely labile: Corbett, D.; Coulton, S.; Southgate, R. Inversion of Configuration at C-8 in the Olivanic Acids: Conversion into the Thienamycins and Other Novel Derivatives. *J. Chem. Soc., Perkin Trans. 1* **1982**, 3011–3016.

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