The Effect of 1,3-Diaryl-[1H]-pyrazole-4-acetamides on Glucose Utilization in ob/ob Mice

Gregory R. Bebernitz,*,† Greg Argentieri,‖ Beverly Battle,‡ Christine Brennan,‡ Bork Balkan,‡,§ Bryan F. Burkey,‖ Michele Eckhardt,‖ Jiaping Gao,‡ Prasad Kapa,# Robert J. Strohschein,† Herbert F. Schuster,† Mary Wilson, and David D. Xu#

Metabolic and Cardiovascular Diseases, Novartis Institute for Biomedical Research, Novartis Pharmaceuticals Corporation, 556 Morris Avenue, Summit, New Jersey 07901

Received January 24, 2001

This article provides evidence of a new class of compounds, 1,3-diaryl-[1H]-pyrazole-4-acetamides, initially identified from their ability to increase glucose transport in an adipocyte and muscle cell line and ultimately demonstrating dramatic glucose lowering in ob/ob mice, a diabetic animal model. The lead compound, **1**, possessed some behavioral-like effects which were removed by structural variation during the course of this investigation. Specifically, **11g** (R1 = meta-CF_3, Ar2 = 4′biphenyl, R3 = diethylamide) illustrated the potency of this series with ED_{50} values for glucose lowering in ob/ob mice of 3.0 mg/kg/day. Concomitant with its effect on glucose lowering, **11g** also caused a 50% reduction in insulin levels consistent with an agent that increases whole body insulin sensitivity. **11g** showed favorable pharmacokinetic data with acceptable absorption, negligible metabolism, and good duration of action. **11g** demonstrated no appreciable adipogenic effect through PPARγ agonism, a characteristic of the thiazolidinediones (TZD), and so represents a potentially new class of agents for the treatment of diabetes.

Diabetes mellitus may be categorized into two subclasses: type I, known as insulin dependent diabetes mellitus (IDDM), and type II, noninsulin dependent diabetes mellitus (NIDDM). IDDM accounts for about 10% of all diabetes and results from autoimmune-mediated destruction of insulin-secreting β-cells of the pancreas. In contrast, NIDDM is a chronic and progressive metabolic disorder of carbohydrate and lipid metabolism and accounts for the remaining 90% of diabetes mellitus.[1] It is the fourth leading cause of death in developed countries and affects more than 5% of the world's population (and one in four people over the age of 60). Fewer than half of all diabetics receive treatment and of these only a very small proportion achieve a level of glucose control that is sufficient to avoid the morbidity associated with the disease, namely, macrovascular (coronary artery disease, stroke) and microvascular (retinopathy, neuropathy, nephropathy, and other microangiopathies) complications. In the 10-year diabetes control and complication trial (DCCT)[2] study, it was found that tight control of blood glucose levels reduced the incidence and progression of neuropathy, retinopathy, and nephropathy, each by more than 50% in IDDM patients. Because therapeutic improvements were continuously related to incremental reductions in glycemia (toward normal levels), a new treatment goal of normoglycemia has been established for both IDDM and NIDDM patients. The initial therapy for newly diagnosed NIDDM patients has conventionally been diet and exercise. Although this leads to a marginal improvement in insulin sensitivity and a corresponding reduction in hyperglycemia, this treatment usually fails within a few months due to lack of compliance and further progression of the disease, and an oral antidiabetic agent is prescribed. The standard regimen has consisted of treatment with a member of the class of sulfonylurea drugs that reduce glycemia by inducing the β-cell to release more insulin. However, undesired consequences of prolonged use of sulfonylureas include hypoglycemic episodes, ultimate exhaustion of the β-cell, as well as the long-term angiogenic side-effects that are a result of chronic 24-h exposure to increased insulin levels.[3−5] Agents that reduce the glycemia without these above side effects are highly desirable and have been the aim of many research groups over the past 20 years. Recently, a new class of compounds, the thiazolidinediones (TZD), have reached the market whose effects on glucose levels in the diabetic are manifested through effects on insulin sensitivity in peripheral tissues without directly effecting the output of insulin from the pancreas. Although successful at reducing glucose levels in diabetic patients, troglitazone, the leader in this class, has been withdrawn from the market due to significant and sometimes lethal liver toxicity in a small portion of the population. Other TZDs, such as rosiglitazone and pioglitazone, have been successful in the treatment of diabetes, although there are still some concerns with this class of drugs with respect to a potential for weight gain, edema, and hepatotoxicity.[6] Since the initial discovery of the TZDs, renewed efforts have been directed toward discovering other novel templates with mechanisms that would provide similar or preferably superior overall effects on insulin sensitivity and glucose homeostasis without any accompanying toxicity.[7]

* Address correspondence to Gregory Bebernitz, phone 908-277-7089, fax 908-277-2405, E-mail greg.bebernitz@pharma.novartis.com.
† Department of Medicinal Chemistry.
‡ Department of Pharmacology.
§ Current Address: IDD, 23 Business PK Dr., Branford, CT 06405.
‖ Department of Biochemistry.
Chemical Development.

10.1021/jm010032c CCC: $20.00
Published on Web 07/06/2001

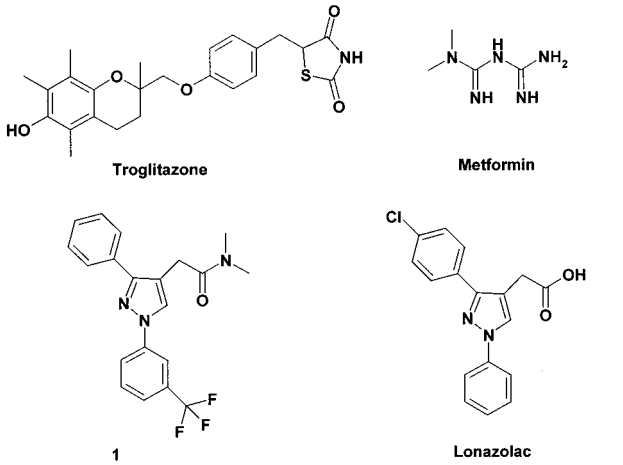


Figure 1. Diabetic agents (troglitazone and metformin), lead pyrazole structure (**1**), Lonazolac.

In an attempt to identify compounds that would increase peripheral glucose utilization, a high-throughput adipocyte screen was used to evaluate our internal compound collection as to their effect on glucose transport. A compound of particular interest, SAH 57-749 (**1**) (Figure 1), was shown to increase [^{3}H] glucose incorporation into lipids in adipocytes in the presence of submaximal concentrations of insulin, suggesting that the compound might increase the sensitivity of the adipocyte to insulin.[8] Furthermore, this compound also demonstrated an increase in glucose transport into L6 myocytes, a cultured muscle cell line. Administration of **1** to ob/ob mice provided a significant reduction in glucose levels over 3 days dosing without any indication of toxicity (see experimental). However, when **1** was administered to normal rats, it was observed to elicit some behavioral-like side-effects. To further examine this, **1** was evaluated both in a CNS receptor panel[9] as well as in a primary observation test (POT)[10] prior to consideration as a lead. No effects were observed in the receptor panel at concentrations up to 10^{-6} M. However, pronounced effects were demonstrated in the POT in both rats and mice at doses of 10, 60, and 360 mg/kg, the most notable effect being a reduction in motor activity and locomotion. These effects eliminated **1** as a potential lead for the glucose utilization project, and the goal became to both separate the antihyperglycemic activity of **1** from its undesired behavioral-like effects as well as improve the potency of the compound as compared to **1**. Early on in this evaluation, it was found that the magnitude of the response in the in vitro assays did not always directly correlate with the magnitude of the response obtained in the in vivo assay, an observation others have previously noted.[11] For this reason, compounds were evaluated based initially on lack of any behavioral-like effects using an abbreviated POT in normal rats followed then by evaluation of their potential for glucose lowering in a diabetic animal model, ob/ob mice.

The initial strategy comprised a traditional medicinal chemistry approach involving variations in both the electronic and steric properties of the appendages off the central pyrazole nucleus of **1**. This evaluation protocol allowed an understanding of not only the compound's ability to lower glucose levels in diabetic animals, but also provided an initial measure of pharmacokinetic properties including bioavailability, duration of action up to 8 h, and a general readout of overt toxicities, an effect of major concern.

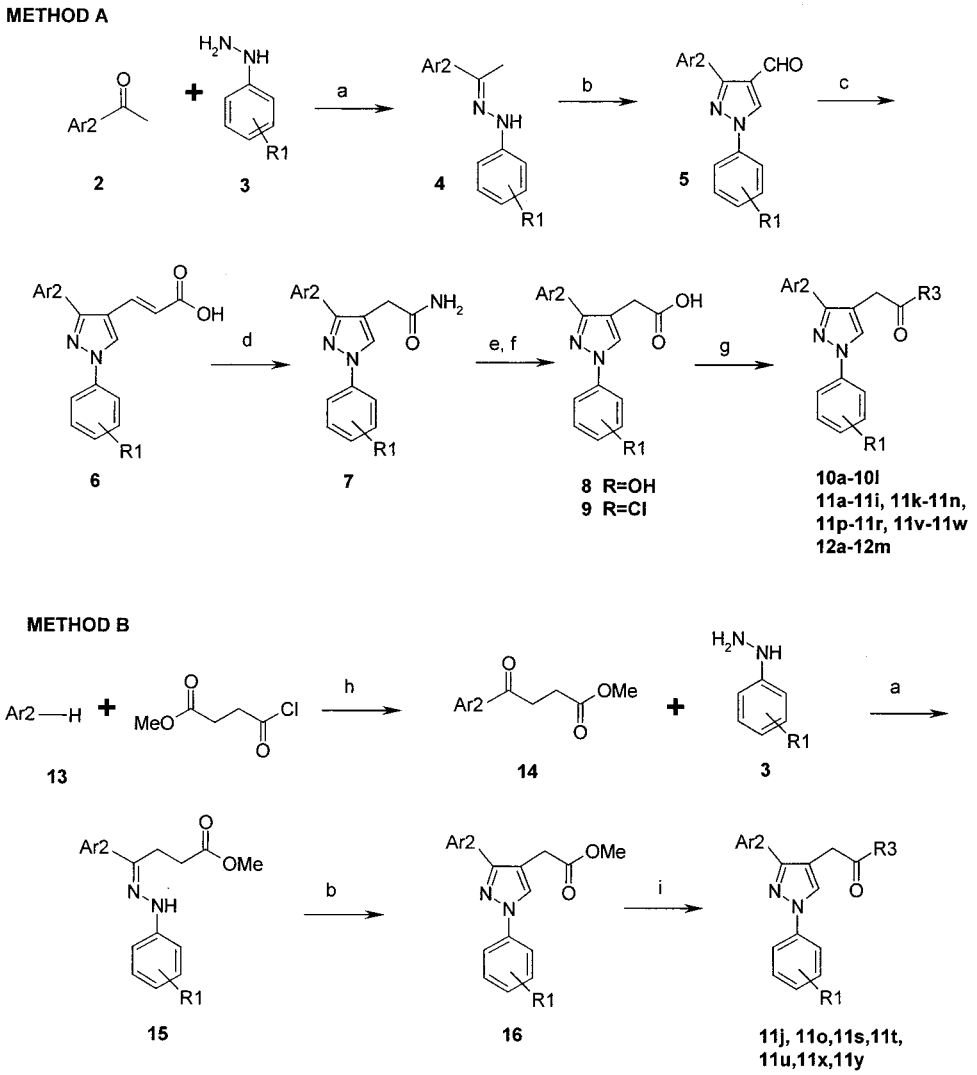
A number of pyrazole compounds have been sited in the literature that elicit anti-hyperglycemic effects including: 1,3-disubstituted pyrazoles,[12] 1,3,5-trisubstituted pyrazoles,[13] and pyrazolones.[14] Structurally though, the compounds described herein have much closer similarity to a body of work performed around nonsteroidal antiinflammatory drugs (NSAID) as exemplified by lonazolac[15] (Figure 1). Amid concerns that the anti-hyperglycemic activity of this series could be related to their antiinflammatory activity, a number of NSAIDs were evaluated in vitro in L6 myocytes for their effects on glucose utilization and showed reduced rather than increased glucose transport and utilization[16] in this cell line.

Chemistry

Variation at the 1- and 3-positions of the pyrazole nucleus generally required an individual synthesis for each desired template. Two somewhat overlapping methods (methods A and B) were utilized to assemble the pyrazole nucleus as shown in Scheme 1. For method A, the synthesis commonly began with commercially available ketones **2** and hydrazines **3** as a starting point. Condensation between a ketone and hydrazine provided a hydrazone **4** in high yields in most cases. The Vilsmeier−Haack reaction using 2.5 equiv of reagent performed a double addition of reagent to afford, ultimately after hydrolysis, the desired cyclized aldehydes, **5** in 80−90% yield.[17] Ortho-substituted phenylhydrazines, however, were not applicable via this route as the condensations either did not lead to product or proceeded in poor yields. It was crucial in this reaction to allow aqueous hydrolysis of the Vilsmeier adduct to proceed to completion, or partial hydrolysis products would complicate the workup and significantly reduce the overall yield. Condensation of **5** with malonic acid provided the unsaturated β-substituted acrylic acid **6** in high yield. Treatment of **6** with sulfur and ammonium hydroxide in a pressure vessel under the Willgerodt−Kindler reaction proceeded with loss of a carbon as a result of these conditions to afford acetamide, **7**. In general the Willgerodt−Kindler reaction conditions allow for carbonyl walk and in the specific case of aryl alkyl ketones afford ultimately terminal carboxamides without any loss of carbon atoms.[18] However, in the case of β-substituted acrylic acids such as **6**, aryl acetamides are efficiently produced.[19] Acetamide **7** was hydrolyzed under acidic conditions to provide the acid, **8**. Compound **8** is then converted in two steps to the desired amide products **10**−**12**. Overall yields over seven steps ranged from 47 to 90%. Although the reaction sequence is somewhat long and linear with few common intermediates, each reaction step is worked up by simply quenching into water and filtering off the product. Method B allowed incorporation of the acetic acid side chain into the template prior to the Vilsmeier−Haack reaction and so shortened the reactions scheme by 2−3 synthetic steps. Whereas the reaction sequence was streamlined in comparison to method A, its usefulness was dependent on the ease of purification of ester product, **14**.

The various hydrazines and methyl ketones are all commercially available with the exception of **2k** which

Scheme 1. Synthetic Methods for the Preparation of Pyrazoles[a]



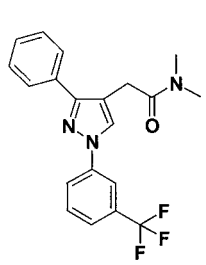
[a] The methods utilized here for the preparation of the pyrazole products are general. Please refer to the Tables 1, 2, and 3 for the specific substitution pattern of the various starting materials as well as the method used for preparation. Conditions: (a) AcOH, water; (b) $POCl_3$, DMF; (c) malonic acid, piperidine, pyridine, reflux; (d) sulfur, p-dioxane, NH_4OH, bomb; (e) AcOH, H_2SO_4, water, reflux; (f) $SOCl_2$, DMF(cat), CH_2Cl_2; (g) R3-NH_2 (or other nucleophile), CH_2Cl_2; (h) $AlCl_3$, 1,1,2,2-tetrachloroethane (i) R3NH_2, $AlCl_3$, CH_2Cl_2.

was prepared under standard Friedel Crafts acylation conditions. **13o** was prepared from fluorene by exhaustive alkylation using methyl iodide and KHMDS. In addition, the extended esters **14j**,**o**,**s**,**t**,**u**,**x**, **and y** used in method B were prepared by the direct Friedel Crafts acylation of the corresponding aromatic precursor, **13**, under standard conditions using methyl 4-chloro-4-oxobutyrate. Oxidation of the methylene position of the fluorenyl side chain of **11n** was carried out using $KMnO_4$ to afford **11r**. Further reduction of the carbonyl of **11r** with $NaBH_4$ and subsequent acylation with acetic anhydride afforded **11p**. Alternatively, addition of methylmagnesium chloride to **11r** provided **11q**.

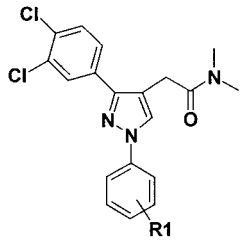
Results and Discussion

Each compound was evaluated first in a normal behavioral rat model under the standard protocols of a CNS - primary observation test (POT).[10] Observations were recorded at 0.5, 1, 2, and 3 h after oral dosing and were based on 10 indices of behavioral activity including: mortality, diarrhea, lacrimation, locomotion, piloerection, twitches, tremors, body position, respiratory rate, and temperature. Although we expected the behavioral-like effects to eliminate a majority of the compounds, we were surprised to find these effects in only a few analogues. In short, the only compounds that exhibited the behavioral-like side effects were various amides of **1** and the phenol ester of **8**. In general, substitution of the aromatic of Ar2 with any substituent resulted in elimination of the behavioral-like activity.[20] Although glucose levels were not expected to change in this normoglycemic rat model, they were also recorded at 3 h postdose to verify that any noted behavioral effects were not the result of significant aberrations in glucose levels either hypoglycemic or hyperglycemic. No statistically significant changes in glucose levels were found for any of the compounds tested in this model including the lead compound, **1**, even considering the high dose of compound administered (1 mmol/kg). Compounds that exhibited no behavioral changes in normal animals were then evaluated for their effects on glucose lowering in ob/ob mice, a diabetic animal model. This entailed dosing the animals once a day for

Table 1. Substitution at the R_1 Position



1



10a-m

cmpd #	R_1 position	Syn method	MP (°C)	empirical formula[a]	ob/ob mice % efficacy[c] dose mg/kg	day 3 4 h	day 3 8 h
1	3-CF_3	A	110−112	$C_{20}H_{18}N_3F_3O$	300	71[d]	36[d]
10a	3-CF_3	A	108−109	$C_{20}H_{16}Cl_2F_3N_3O$	300	NS	63[d]
10b	3-F[b]	A	102−105	$C_{21}H_{18}Cl_2FN_3O$	300	74[d]	75[d]
10c	3-NO_2	A	194−195	$C_{19}H_{16}Cl_2N_4O_3$	100	−72[d]	−88[d]
10d	3-CH_3	A	100−102	$C_{20}H_{19}Cl_2N_3O$	100	NS	NS
10e	4-OMe	A	109.5−111	$C_{20}H_{19}Cl_2N_3O_2$	300	39[d]	35[d]
10f	4-Cl	A	138−139	$C_{19}H_{16}Cl_3N_3O$	300	47[d]	52[d]
10g	4-F	A	141−143	$C_{19}H_{16}Cl_2FN_3O$	300	84[d]	39[d]
10h	4-*tert*-butyl	A	141−144	$C_{23}H_{25}Cl_2N_3O$	100	NS	NS
10i	4-CF_3	A	128−129	$C_{20}H_{16}F_3Cl_2N_3O$	300	45[d]	37[d]
10j	3,4-Cl_2	A	144−145	$C_{19}H_{15}Cl_4N_3O$	300	34[d]	53[d]
10k	3,5-Cl_2	A	145−146	$C_{19}H_{15}Cl_4N_3O$	300	52[d]	NS
10l	3,5-$(CF_3)_2$	A	120.5−122	$C_{21}H_{15}Cl_2F_6N_3O$	300	NS	NS
10m	3,5-$(CH_3)_2$	A	127−128	$C_{21}H_{21}Cl_2FN_3O$	300	NS	NS

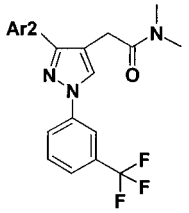
[a] Analytical results were within 0.4% of the theoretical value. [b] Results shown are for the pyrrolidine amide rather than the dimethylamide. Ref 42. [c] Values are presented as % efficacy where 100% efficacy results in normalization of the blood glucose levels to that of normal mice. NS = nonsignificant. [d] $p < 0.05$.

3 days and obtaining glucose levels at 2 and 4 h on day 1 and 2, 4, and 8 h postdose on day 3 (see Experimental Section).

Variations at the 1, 3, and amide positions of the pyrazole (R1, Ar2, and R3, respectively) were each done independently due in part to the linearity of the synthesis (Tables 1, 2, and 3, respectively). Although the specific target for these compounds is unknown, the potency and efficacy of these compounds in vivo would argue for a specific target, and this premise forms the basis for the discussion to follow. For variation of substituents on the aromatic group at the 1-position (R1, Table 1), both electron withdrawing and releasing groups appeared beneficial without any apparent preference in terms of 3- or 4-position on the aromatic ring. Simple alkyl substitution proved generally inadequate,[21] while substitution with the 3-nitro (**10c**) actually caused the opposite and undesired effect of hyperglycemia.

As compared to substitution at R1, substitution at Ar2 resulted in more substantial improvement in activity (Ar2, Table 2). Although both electron withdrawing (**10a**, **11a**) and electron releasing groups (**11b**, **11f**) appeared beneficial, bulky and flexible substituents placed at the 4-position of the aromatic (**11c**−**11e**) resulted in a loss of activity. In light of these findings, it was interesting that substitution of an aromatic group at the 4-position to afford a biphenyl moiety (**11g**) resulted in an improvement in activity. The position of the aromatic substitution was shown to be important with the para-phenyl substitution providing much better activity than the corresponding meta-substituted (**11i**) or ortho-substituted (**11h**) adducts. Immobilization of the two aromatic groups of biphenyl by means of a cyclic fluorenyl analogue (**11n**) proved especially interesting with an increase in potency of 26-fold. Two parameters that might account for the noted difference between **11g** and **11n** are electronics and conformational flexibility. It has been shown by various techniques[22] that whereas the twist angle between phenyl groups in the fluorenyl group is expectedly 0°, the twist in the biphenyl is approximately 26−30° indicating that these two substituents have a somewhat different orientation in space. In addition, substituent stability studies have demonstrated[23] that more electronic character of the substituents is transferred to the adjacent aromatic when the two aromatics are planar as is the case for the fluorenyl system than would be the case for the biphenyl. As variation in electronic character at the R_2 position had no preferential effect on activity, this may suggest that the planar orientation of the fluorenyl moiety[24] might be the primary determinant of enhanced activity perhaps through some preferential effect on pi stacking. We therefore examined variations in Ar2 that would challenge the ability of this substituent to efficiently pi stack. Functionalization of the 9-position of the fluorenyl group with hydrogen bond donors and acceptors (**11o**−**11r**) did not compromise their activity, suggesting that the binding pocket does allow some flexibility. However, addition of a third aromatic group as in **11j** resulted in loss of activity. As it had been demonstrated (**11g**−**11i**) that para-substitution of the second aromatic group of the biphenyl was necessary for activity, we further investigated what effect changes in orientation would have on activity. Substitution of a phenanthrene for Ar2 with attachment at either the 2- or 9-position (**11v** vs **11w**) provided additional support for this preferred para-aromatic orientation. Although both compounds (**11v** and **11w**) possessed similar physical and pharmacokinetic properties,[25] only **11v** was found to be active. On the basis of further substitutions

Table 2. Substitution at the Ar2 Position



11a-y

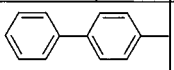
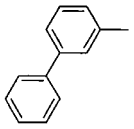
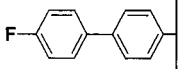
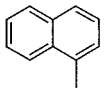
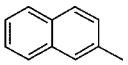
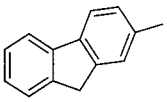
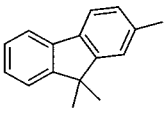
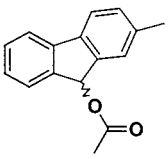
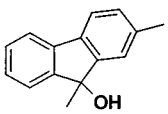
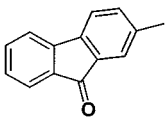
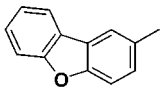
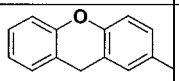
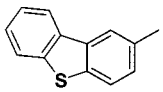
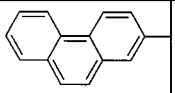
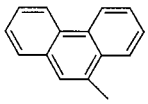
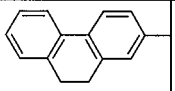
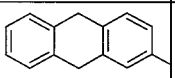
Cmpd #	Ar2 position	Syn method	MP (°C)	Empirical Formula [a]	Ob/ob mice % Efficacy [b]		
					Dose mg/kg	Day 3 4h	Day 3 8h
11a	4-chlorophenyl	A	132-133	$C_{20}H_{17}ClF_3N_3O$	300	NS	78*
11b	3,4-dimethoxyphenyl	A	49-63 glass	$C_{22}H_{22}F_3N_3O_3$	100	NS	85*
11c	4-benzylphenyl	A	114.5-116	$C_{27}H_{24}F_3N_3O$	100	NS	NS
11d	4-phenoxyphenyl	A	120-121	$C_{26}H_{22}F_3N_3O_2$	100	NS	NS
11e	4-t-butylphenyl	A	94-95	$C_{24}H_{26}F_3N_3O$	100	NS	NS
11f	4-tolyl	A	98-99.5	$C_{21}H_{20}N_2F_3O$	300	58*	36*
11g		A	125-126	$C_{26}H_{22}F_3N_3O$	100	84*	69* ED_{50}=1.3 mg/kg/day
					30	80*	94*
11h		A	50-60d	$C_{26}H_{22}F_3N_3O$	100	NS	NS
							
11i		A	foam	$C_{26}H_{22}F_3N_3O$	100	NS	NS
11j	4,4'-triphenyl	B	180	$C_{32}H_{26}F_3N_3O$	100	NS	NS
11k		A	122-125	$C_{26}H_{21}F_4N_3O$	100	NS	81*
11l		A	75-80	$C_{24}H_{20}F_3N_3O$	100	NS	NS
11m		A	141-142	$C_{24}H_{20}F_3N_3O$	100	NS	NS
11n		A	99-101	$C_{27}H_{22}F_3N_3O$	100	87*	57* ED_{50}=0.05 mg/kg/day
					30	113*	115*
11o		B	98-101	$C_{29}H_{26}F_3N_3O$	100	67*	83*
11p		A[c]	191-192	$C_{29}H_{24}F_3N_3O_3$	100	95*	108*

Table 2 (Continued)

Cmpd #	Ar2 position	Syn method	MP (°C)	Empirical Formula [a]	Ob/ob mice % Efficacy [b]		
					Dose mg/kg	Day 3 4h	Day 3 8h
11q		A[c]	170-172	$C_{28}H_{24}F_3N_3O_2$	100	73*	64*
11r		A[c]	144-146	$C_{27}H_{20}F_3N_3O_2$	100	107*	78*
11s		B	190-191	$C_{26}H_{20}F_3N_3O_2$	100	NS	NS
11t		B	168-169.5	$C_{27}H_{22}F_3N_3O_2$	100	65*	96*
11u		B	183-184	$C_{26}H_{20}N_3F_3OS$	100	59*	NS
11v		A	130-132	$C_{28}H_{22}F_3N_3O$	100	90*	71*
11w		A	141-143	$C_{28}H_{22}F_3N_3O$	100	NS	NS
11x		B	133-134	$C_{28}H_{24}F_3N_3O$	100	87*	111*
11y		B	142.5-144	$C_{28}H_{24}F_3N_3O$	100	79*	70*

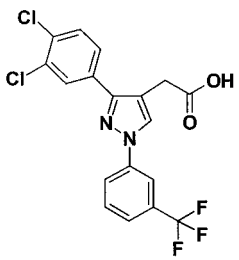
[a] Analytical results were within 0.4% of the theoretical value. [b] Values are presented as % efficacy where 100% efficacy results in normalization of the blood glucose levels to that of normal mice. [c] Parent compound is prepared by the indicated method. However further chemistry is required to prepare the shown product as described in the Experimental Section. NS= nonsignificant. *$p < 0.05$

around Ar2, it was concluded that, in general, compounds that could adopt a para-biphenyl orientation (**11g**, **11k**, **11n**, **11o**−**11r**, **11v**, **11x**) would be active while compounds that could not adopt this orientation (**11h**, **11i**, **11l**, **11m**, **11s**, **11w**) would be inactive. However, two compounds, **11t** and **11y**, which did not conform to this para-biphenyl paradigm, were also active, suggesting ultimately that some pi stacking motif or pharmacokinetic property may be responsible for the activity profile observed for substitution at Ar2.

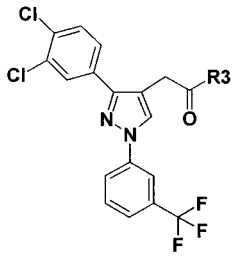
Variation of the amide portion (R3) suggested that tertiary amides were generally preferred, with secondary amides (**12h**) and esters or acids (**12j** and **8**) being inactive. Interestingly, whereas the pyrrolidine and dimethyl amides were found to have similar activity in most cases (i.e., **12a** and **12c**) other structurally similar amides (**12d** and **12b**, respectively) were not active. Substituted piperazine and dibenzyl amides (**12g** and **12m**) also exhibited some interesting activity although these substitutions dramatically increased their lipophilic character (cLogP 7−9).

The highly lipophilic character of this series of compounds would be considered a significant development hurdle. Even though **11g** and **11n** had high estimated cLogP′ values[26] (5.9 and 5.8, respectively) and in vitro penetration values,[27] which would indicate poor bioavailability, absolute oral bioavailability in normal rats was determined on average to be quite good (i.e., 25−30% from normal aqueous CMC suspensions). Addition of formulation adjuvants[28] used typically for water insoluble compounds allowed for a substantial increase in bioavailability (50−60% range) for **11g** with a concomitant 2.5-fold improvement in EC_{50} to 0.56 mg/kg (Figure 3). Although **11n** already demonstrated

Table 3. Substitution at the R_3 Position



8



12a-m

cmpd #	R_3 position	Syn method	MP (°C)	empirical formula[a]	ob/ob mice % efficacy[b] dose mg/kg	day 3 4 h	day 3 8 h
8		A	194−197	$C_{18}H_{11}Cl_2F_3N_2O$	300	NS	NS
12a	diethylamine	A	92−93	$C_{22}H_{20}Cl_2F_3N_3O$	300	45[c]	50[c]
12b	piperidine	A	137−138	$C_{23}H_{20}Cl_2F_3N_3O$	300	NS	NS
12c	pyrrolidine	A	124−125	$C_{22}H_{18}Cl_2F_3N_3O$	300	72[c]	76[c]
12d	morpholine	A	178−179	$C_{22}H_{18}Cl_2F_3N_3O_2$	300	NS	NS
12e	4-methyl piperazine	A	158−159	$C_{23}H_{21}Cl_2F_3N_4O$	300	54[c]	NS
12f	4-phenylpiperazine	A	171−173	$C_{28}H_{23}Cl_2F_3N_4O$	100	NS	42[c]
12g	4-diphenylmethyl piperazine	A	123−125	$C_{35}H_{29}Cl_2F_3N_4O$	100	NS	45[c]
					30	NS	48[c]
12h	aniline	A	161−166	$C_{24}H_{16}Cl_2F_3N_3O$	300	NS	NS
12i	*N*-methylaniline	A	104−105	$C_{25}H_{18}Cl_2F_3N_3O$	300	57[c]	NS
12j	ethanol	A	66−67	$C_{20}H_{15}Cl_2F_3N_2O_2$	300	NS	NS
12k	methoxyamine	A	212−214	$C_{19}H_{14}Cl_2F_3N_3O_2$	300	48[c]	35[c]
12l	hydroxylamine	A	200(D)	$C_{18}H_{12}Cl_2F_3N_3O_2$	300	NS	NS
12m	dibenzylamine	A	102−103	$C_{32}H_{24}Cl_2F_3N_3O$	100	52[c]	49[c]

[a] Analytical results were within 0.4% of the theoretical value. [b] Values are presented as % efficacy where 100% efficacy results in normalization of the blood glucose levels to that of normal mice. NS= nonsignificant. [c] $p < 0.05$.

remarkable potency (ED_{50} = 0.05 mg/kg), a corresponding increase in bioavailability was not seen however for **11n** under similar dosing conditions due to its substantially reduced solubility in the microemulsion vehicle itself.[29] Although SAR studies suggested that some measure of lipophilicity was necessary for the desired in vivo activity, a plot of activity versus cLogP did not provide any meaningful correlation.[30]

It has been suggested that the thiazolidinedione's effect on moderating glucose levels and insulin sensitivity is through effects on adipocyte differentiation which are mediated through binding to the PPAR-γ receptor, a key adipogenic transcription factor.[31] It has also been shown that the relative affinity of these ligands to the PPAR-γ receptor is predictive of their corresponding anti-hyperglycemic effects in vivo.[32] This mechanism has resulted chronically in increased adiposity in animal models[33] as well as in patients.[34] For this reason, we evaluated **11g** for its effects on adipocyte differentiation. As evident from Figure 2, whereas troglitazone exhibited enhanced adipocyte differentiation, metformin and **11g** had no effect. This was a very positive finding for this series and supported that these compounds were providing glucose lowering through some mechanism other than through PPAR-γ receptor activation.

As the mechanistic target for **11g** is unknown, a further evaluation of its pharmacological characteristics was undertaken. **11g** exhibited increased 2-DG uptake into 3T3-L1 adipocytes both in the presence and absence of submaximal insulin.[35] **11g** had no effect on fatty acid oxidation, glucose production, or ATP levels in isolated hepatocytes.[36] In addition to the significant anti-hyperglycemic effect **11g** demonstrated in ob/ob mice, a corresponding 50% reduction in insulin levels[37] was observed consistent with an improvement in overall insulin sensitivity. Both body weights and food intake were reduced in comparison to vehicle matched controls[38] for **11g**. In addition, as these compounds had structural similarities to lonazolac, **11g** was evaluated in a model of acute inflammation (p.o. in rat carrageenan-induced paw oedema) and was weak as compared to the activity of lonazolac and other NSAIDs.[39] The behavioral-like side effects characteristic of **1** were not observed with **11g**. Taken together with its bioavailability (vide supra), duration of action (up to 8 h) and lack of tachyphylaxis,[40] **11g** was considered an attractive candidate for glucose lowering in diabetics.

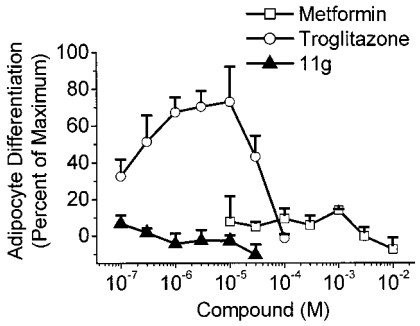


Figure 2. Stimulation of preadipocyte differentiation by antidiabetic agents effects of above listed compounds on 3T3-L1 preadipocyte conversion. Two-day postconfluent 3T3-L1 preadipocytes were placed in DMEM containing 10% FBS and 160 nM insulin. Cells were fed fresh medium and compound every 2 days. On day 7, cells were washed once and fixed in 10% formalin, and lipids were stained with old red O. Cellular old red O content was measured by image analysis and adipocyte differentiation was expressed as a percent maximal adipocyte differentiation stimulated using standard differentiation medium (160 nM insulin, 0.5 mM IBMX, 0.25 μM DEX). Data are represented as mean ± SEM of three independent experiments.

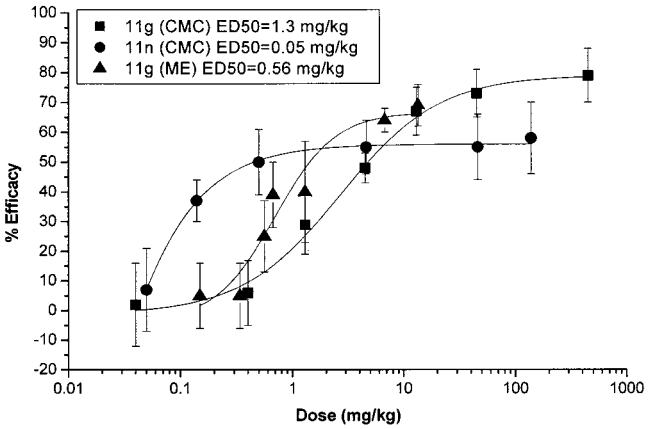


Figure 3. Dose response curves of glucose lowering in ob/ob mice for **11g**, **11g**, (microemulsion) and **11n**. Dose response curves were generated using standard ob/ob mouse protocol as found in Experimental Section at selected doses. Key indicates compound and vehicle in parentheses (CMC = carboxymethylcellulose, ME = microemulsion vehicle as described in text). ED50 values were calculated using a sigmoidal fit within Origin 6.1.

In conclusion, **11g** is a potent and efficacious agent that significantly lowers glucose and insulin levels in an animal model of diabetes (ob/ob mouse) without showing any effect on glucose levels in normal rats. The behavioral effects apparent in the lead structure, **1**, were readily eliminated and this effect seems to have been an aberration not generally observed with the majority of analogues described herein. Finally, these compounds compared favorably in ob/ob mice with marketed drug agents such as metformin (ED_{50} = 128 mg/kg/day) and troglitazone (ED_{50} = 45 mg/kg/day)[41] and thus have the potential to provide a valuable addition to the repertoire of antidiabetic therapies.

Experimental Section

Biological Methods. (a) Preadipocyte Differentiation Assay. Preadipocytes were seeded in 48-well plates at a density of 5000 cells/0.5 mL of predifferentiation medium (high glucose (25 mM) DMEM (Gibco, Gaithersburg, MD), containing 10% newborn calf serum (Gibco), 50 units/mL penicillin (Gibco), and 50 mg/mL streptomycin sulfate (Gibco)) and maintained in a 7% CO_2 humidified atmosphere at 37 °C. On day 7, confluent preadipocytes were changed to postdifferentiation medium (high glucose DMEM containing 10% fetal bovine serum (FBS, Gibco), 50 units/mL penicillin, 50 mg/mL streptomycin sulfate, 10 mg/mL D-biotin (Sigma, St Louis, MO), and 10 mg/mL pantothenic acid (Sigma)). To induce differentiation, test compounds were added on day 7 to postdifferentiation medium or postdifferentiation medium containing 1 mg/mL porcine pancreas insulin (Sigma, #I-3505), where the final DMSO concentration in the medium was 0.2%. Cells were refed fresh test medium every 2 days. As a control, full differentiation was induced on day 7 by treating cells with postdifferentiation medium containing 0.5 mM 3-isobutyl-1-methylxanthine (IBMX, Sigma), 1 mg/mL porcine pancreas insulin (Sigma, #I-3505), and 0.25 mM dexamethasone (Sigma). After 2 days, control cells were washed once with Dulbecco's PBS (D-PBS) and exposed to the above medium without IBMX and dexamethasone for two additional days. Thereafter, fully differentiated control cells were maintained in postdifferentiation media in the absence of insulin, dexamethasone, or IBMX. On day 14, cell monolayers were fixed for 1 h in 10% buffered formalin, and cellular lipid was stained with oil red O. Accumulation of oil red O was measured using the IBAS image analysis software (Kontron, Munich, Germany) as the mean gray density of light transmission using a Nikon Microphot.fxa microscope with an attached Panasonic CB50 video camera. Data are expressed as a percent of maximal adipocyte differentiation obtained using a standard differentiation medium and are the mean ± SEM of three independent experiments.

(b) Primary Observation Test (POT). Normal male Sprague Dawley rats were housed in groups under normal light cycle (lights on at 6 am) with ad lib access to chow (Purina rodent chow) and tap water. The animals (n = 3 per group) were orally dosed with 1000 μmol/kg in vehicle (carboxymethylcellulose (CMC, 0.5%) with Tween-80 (0.2%)) in the postabsorptive state (food removed at 6 am dosing at 10 am). SDZ 57-749(**1**) was included in each experiment as a positive control. Independent behavioral observations were made by two investigators at 0.5, 1.2, and 3 h postdose. These observations were based on 10 indices of behavior including: mortality, diarrhea, lacrimation, locomotion, piloerection, twitches, tremors, body position, respiratory rat,e and temperature. One blood sample was obtained via tail nick for the determination of blood glucose concentrations at 3 h postdose.

(c) Chronic Glucose Lowering in ob/ob Mice. Adult male C57BL ob/ob mice were housed four per cage in hanging wire bottom cages with standard laboratory conditions and a 12:12 h light/dark cycle. Food (Purina rodent chow) and water were available ad libitum. For blood sampling, the mice were placed in a restrainer and a drop of blood was obtained through a nick in the tail. Blood glucose concentrations were determined in 10 mL of blood using a YSI27 analyzer (Yellow Springs Instruments). The protocol for compound evaluation was as follows: The mice were distributed into groups (n = 6 per group) matched for blood glucose levels on day 0. The animals were dosed (p.o.) in the mornings of days 1−3 with vehicle (CMC, 0.5% with Tween-80 (0.2%)) or compound in vehicle. Blood glucose concentrations were monitored at 2 and 4 h post-dose on day 1 and 2, 4, and 8 h post-dose on day 3. Behavioral observations were made before dosing on day 2 and on day 3 before the 4 h sampling. Results are expressed as percent efficacy, that is the ability of the compound to normalize blood glucose levels to 100 mg/dL and calculated by the formula $\{1 - ([\text{glucose}]_{\text{compound}} - 100)/([\text{glucose}]_{\text{vehicle}} - 100)\} \times 100$. Animals that displayed glycemia of less than 150 mg/dL on day 0 (basal) were considered not diabetic and were excluded from the study. Statistical significance ($p < 0.05$) was evaluated by a t-test comparison to the appropriate sample in vehicle-treated animals.

For clarity, only data from day 3 @ 4 and 8 h are shown. If the results were not significant, NS is displayed in the column.

Chemical Methods

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR were recorded on a Bruker AC300 MHz NMR using tetramethylsilane (TMS) as an internal standard and are reported in ppm (δ). Mass spectra were performed on a Finnigan Mat 4600 spectrometer. Elemental analysis were performed with a Carlo Erba CHNS-O EA 1108 elemental analyzer. Data are within 0.4% of theoretical values unless otherwise indicated. All compounds were routinely checked by TLC with Macherey-Nagel Polygram SIL G/UV_{254} plates. Yields are of purified products and were not optimized.

Method A: General Procedures for the Preparation of Pyrazole. Preparation of 3-[1,1′-biphenyl]-4-yl-*N*,*N*-dimethyl-1-[3-(trifluoromethyl)phenyl]-1H-pyrazole-4-acetamide (11 g): (a) 1-[1,1′-Biphenyl]-4-yl-, [3-(trifluoromethyl)phenyl]hydrazone, (1E) ethanone (4g). To 4-phenylacetophenone (0.1 mol, 19.89 g) in 200 mL of acetic acid and 10 mL of water was added 3-trifluoromethylphenylhydrazine (0.1 mol, 19.4 g). Shortly after addition, a solid mass formsed and acetic acid was added to maintain stirring for 15 h. The reaction mixture was diluted with an additional 50 mL of water and subsequently filtered and the solid was dried in vacuo to afford 33.1 g of crystalline product **4g** (93% yield): mp 130−133 °C; ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3H), 7.38 (m, 3H),

7.47 (m, 4H), 7.63 (d, J = 9.7 Hz, 4H), 7.87 (d, J = 9.7 Hz, 2H); MS (DCI, isobutane) *m*/*z* (rel intensity) 355 (100).

(b) 3-[1,1′-Biphenyl]-4-yl-1-[3-(trifluoromethyl)phenyl]-1H-pyrazole-4-carboxaldehyde (5g). Phosphorus oxychloride (0.21 mol, 20 mL) was added to 150 mL of DMF at 0 °C and stirred for 30 min. **4g** (0.094 mol, 33.1 g) was added as a solid slowly to this mixture and stirred for 15 h. The crude reaction was then quenched into 1 L of water and stirred for an additional 15 h. The resulting solid was filtered, dissolved in methylene chloride, and dried over $MgSO_4$, filtered, and evaporated to a crude solid that was crystallized from ether/hexane (5:1) to afford 30.4 g of **5g** in 83% yield: mp 190−191 °C; ^{1}H NMR ($CDCl_3$) δ 7.39 (m, 1H), 7.45 (t, J = 7.5 Hz, 2H), 7.66 (d, J = 6.75 Hz, 4H), 7.73 (d, J = 8.25 Hz, 2H), 7.93 (d, J = 8.25 Hz, 2H), 8.02 (m, 1H), 8.17 (s, 1H), 8.63 (s, 1H), 10.15 (s, 1H), MS (DCI, isobutane) *m*/*z* (rel intensity) 393 (100).

(c) 3-[3-[1,1′-Biphenyl]-4-yl-1-[3-(trifluoromethyl)phenyl]-1H-pyrazol-4-yl]-, (2E)-2-propenoic acid (6g). To **5g** (0.077 mol, 30.35 g) in 175 mL of pyridine and 1 mL of piperidine was added malonic acid (0.16 mol, 16.9 g), and the mixture heated at 90 °C for 12 h. An additional portion of malonic acid (0.028 mol, 4 g) was added and heated at 90 °C for 3 h. The temperature was then raised to 135 °C and heating was continued for 3.5 h. The reaction was cooled to ambient temperature and then quenched into a mixture of HCl (conc) and ice. After stirring the sample for 2 h, the resulting solid was filtered, washed 3 times with water, and dried in vacuo to afford **6g** (33.4 g, 99%): mp >250 °C; ^{1}H NMR (d_6DMSO) δ 6.51 (J = 16.5 Hz, 1H), 7.39 (m, 1H), 7.45 (t, J = 7.5 Hz, 2H), 7.59 (d, J = 16.5 Hz, 1H), 7.75−7.9 (m, 8H), 8.30 (m, 2H), 9.41 (s, 1H); MS (DCI, isobutane) *m*/*z* (rel intensity) 435 (100).

(d) 3-[1,1′-Biphenyl]-4-yl-1-[3-(trifluoromethyl)phenyl]-1H-pyrazole-4-acetamide (7g). To **6g** (0.077 mol, 33.4 g) in 25 mL of ammonium hydroxide (conc) and 75 mL of 1,4-dioxane was added sulfur (0.5 mol, 16 g) and the resulting slurry was heated in a pressure vessel to 175 °C for 15 h. The reaction vessel was cooled, the pressure was released, and the reaction mixture was quenched into water and stirred for 2 h. Filtration afforded **7g** as an off-white solid (36.5 g) that was used directly in the next reaction: mp 241−243 °C; ^{1}H NMR (d_6DMSO) δ 3.57 (s, 2H), 7.04 (s, 1H), 7.40 (t, J = 7.4 Hz, 1H), 7.50 (m, 3H), 7.69 (d, J = 8 Hz, 1H), 7.75 (m, 3H), 7.78 (d, J = 8 Hz, 2H), 7.86 (d, J = 8 Hz, 2H), 8.22 (d, J = 8 Hz, 1H), 8.24 (s, 1H), 8.64 (s, 1H); MS (DCI, isobutane) *m*/*z* (rel intensity) 422 (100).

(e) 3-[1,1′-Biphenyl]-4-yl-1-[3-(trifluoromethyl)phenyl]-1H-pyrazole-4-acetic acid (7g). The crude amide **7g** (0.077 mol, 36.5 g) was suspended in glacial acetic acid (200 mL) and 50% sulfuric acid (140 mL) was refluxed for 2 h and then poured into 2 L of water. The resulting solids were filtered, washed with water until the filtrate was neutral, and subsequently dried in vacuo to afford acid **8g** (31.3 g, 97%): ^{1}H NMR (CD_3OD) δ 3.76 (s, 2H), 7.37 (t, J = 7 Hz, 1H), 7.48 (t, J = 7 Hz, 2H), 7.6−7.8 (m, 8H), 7.96 (d, J = 7 Hz, 1H), 8.06 (s, 1H), 8.13 (s, 1H); MS (DCI, isobutane) *m*/*z* (rel intensity) 423 (100).

(f) 3-[1,1′-Biphenyl]-4-yl-*N*,*N*-dimethyl-1-[3-(trifluoromethyl)phenyl]- 1H-pyrazole-4-acetamide (11g). 8g (0.035 mol, 15 g) was dissolved in methylene chloride (300 mL) with a catalytic amount of DMF (0.5 mL) and thionyl chloride (0.35 mol, 26 mL) was added and stirred for 4 h before the reaction was evaporated and pumped on in vacuo for 12 h. The resulting acid chloride, **9g** was dissolved in methylene chloride (200 mL) and cooled to 0 °C. Dimethylamine (0.1 mol, 4.5 g) was condensed into cold methylene chloride (20 mL) and added to the acid chloride and stirred warming to ambient temperature over 15 h. The reaction mixture was evaporated and then partitioned between ethyl acetate and water. The combined organic layer was dried over $MgSO_4$, filtered, and evaporated to give the amide as a tan solid. The crude material was chromatographed on silica eluting with hexane to 50% EtOAc/hexane to afford **11g** (12.7 g, 81%): mp 125−126 °C; ^{1}H NMR ($CDCl_3$) δ 2.98 (s, 3H), 3.01 (s, 3H), 3.78 (s, 2H), 7.37 (t, J = 7 Hz, 1H), 7.47 (t, J = 8 Hz, 2H), 7.52 (d, J = 7 Hz, 1H), 7.57 (t, J = 7 Hz, 1H), 7.65 (d, J = 7 Hz, 2H), 7.72 (q, J = 9 Hz, 4H), 7.96 (d, J = 8 Hz, 1H), 8.07 (s, 1H), 8.12 (s, 1H); MS (DCI, isobutane) *m*/*z* (rel intensity) 450 (100). Anal. ($C_{26}H_{22}F_3N_3O$), C,H, N.

9,9-Dimethylfluorene (13o). Fluorene (50 mmol, 8.3 g) was dissolved in THF (150 mL) and cooled to 0 °C. KHMDS (50 mmol, 100 mL of 0.5 M solution/toluene) was added dropwise at 0 °C, and then the reaction mixture was allowed to warm to RT for 30 min. The mixture was then cooled to 0 °C and methyl iodide (50 mmol, 3.2 mL) was added and stirred for 30 min. This cycle was repeated with an additional amount of KHMDS (50 mmol, 100 mL of 0.5 M solution/toluene) and subsequently methyl iodide (50 mmol, 3.2 mL) and the reaction mixture was allowed to warm to RT overnight. The reaction mixture was quenched into aqueous NaH_2PO_4 and extracted with MTBE. The organic extracts were dried and evaporated to afford **13o** as a waxy solid (9.8 g, 100%): ^{1}H NMR ($CDCl_3$) δ 1.50 (s, 6H), 7.3 (m, 4H), 7.44 (m, 2H), 7.70 (m, 2H); MS (DCI, isobutane) *m*/*z* (rel intensity) 195 (100).

Method B: General Procedure for the Preparation of Pyrazoles. (a) Step A − Preparation of the Acylated Precursor 14: 9,9-Dimethyl-γ-oxo-9H-fluorene-2-butanoic acid, Methyl Ester (14o). To 9,9-dimethylfluorene (**13o**) (50 mmol, 9.7 g) and methyl 4-chloro-4-oxobutyrate (51 mmol, 6.3 mL) in 1,1,2,2-tetrachloroethane (100 mL) at 0 °C was added $AlCl_3$ (105 mmol, 14 g) portionwise over 10 min. The resulting mixture was stirred for 1 h at 0 °C and 2 h at ambient temperature and then quenched into cold 6 N HCl and extracted 3× with methylene chloride. The combined organic layer was dried over $MgSO_4$ and then evaporated to a crude oil that was chromatographed over silica (20% EtOAc/hexane) to afford **14o** as a thick oil (14 g, 91%): ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 6H), 2.80 (t, J = 7.5 Hz, 2H), 3.40 (t, J = 7.5 Hz, 2H), 3.74 (s, 3H), 7.37 (m, 2H), 7.47 (m, 1H), 7.77 (m, 2H), 8.00 (d, J = 7.5 Hz, 1H), 8.06 (s, 1H); MS (DCI, isobutane) *m*/*z* (rel intensity) 309 (100).

(b) Step B − Preparation of the Pyrazole Product. (1) 3-(9,9-Dimethyl-9H-fluoren-2-yl)-*N*,*N*-dimethyl-1-[3-(trifluoromethyl)phenyl]-1H-pyrazole-4-acetamide (11o). 14o (16.2 mmol, 5 g) was directly treated with 3-trifluoromethylphenylhydrazine (16.4 mmol, 3.21 g) in propionic acid (50 mL) for 4 h before being evaporated to a crude oil. The residue was partitioned between MTBE and water, washed with brine, dried, and evaporated to afford **15o** as a thick oil (7.6 g, 99%). ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 6H), 2.7−2.8 (m, 2H), 2.9−3.15 (m, 2H, E/Z isomers), 3.73 (s, 3H), 6.9−7.9 (m, 11H), 9.1 (bs, 1H) MS (DCI, isobutane) *m*/*z* (rel intensity) 467 (100). The Vilsmeier reagent was prepared by adding $POCl_3$ (86 mmol, 8 mL) to DMF (50 mL) at 0 °C and allowing the mixture to stir and warm to room temperature over 2 h). Crude **15o** (16 mmol 7.6 g) was dissolved in DMF (25 mL), chilled to 0 °C, and added to the Vilsmeier reagent, and the mixture was stirred to 20 h warming to room temperature. The reaction mixture was quenched into water (1 L) and stirred for 15 h. The aqueous layer was decanted and the residue was dissolved in MTBE and washed with brine, dried, and evaporated to an oil. Chromatography on silica (50−80% CH_2Cl_2/pentane) afforded 3-(9,9-dimethyl-9H-fluoren)-2-yl-1-[3-(trifluoromethyl)phenyl]-1H-pyrazole-4-acetic acid, methyl ester **(16o)** (4.6 g, 61%): ^{1}H NMR ($CDCl_3$) δ 1.55 (s, 6H), 3.74 (s, 3H), 3.84 (s, 2H), 6.9−8.2 (m, 12H); MS (DCI, isobutane) *m*/*z* (rel intensity) 477 (100).

Dimethylamine (69 mmol, 3.1 g) was dissolved in toluene at 0 °C before addition of $AlCl_3$ (20 mmol, 2.8 g) portionwise over 10 min. The mixture was stirred for 15 min and then **16o**, dissolved in 15 mL of toluene, was added and the reaction mixture was allowed to warm to RT and stirred for 15 h. The reaction was evaporated, quenched into ice water (300 mL), extracted with EtOAc, and washed with aqueous 1 N HCl and brine. The organic layer was dried and evaporated to a crude oil. The oil was crystallized from MTBE/heptane to afford **11o** (3.05 g, 65%): mp 98−101 °C; ^{1}H NMR ($CDCl_3$) δ 1.54 (s, 6H), 2.95 (s, 3H), 3.01 (s, 3H), 3.77 (s, 2H), 7.29−7.41 (m, 2H), 7.42−7.64 (m, 4H), 7.68−7.86 (m, 3H), 7.97 (d, J = 8 Hz, 1H), 8.08 (s, 1H), 8.14 (s, 1H); MS (DCI, isobutane) *m*/*z* (rel intensity) 490 (100); Anal. ($C_{29}H_{26}F_3N_3O$), C,H,N,F.

3-[9-(Acetyloxy)-9H-fluoren-2-yl]-*N,N*-dimethyl-1-[3-(trifluoromethyl)phenyl]-1H-pyrazole-4-acetamide (11p). To **11n** (0.44 mmol, 0.21 g) in ethanol (10 mL) cooled to 5 °C was added $NaBH_4$. After 1 h, the reaction was evaporated and then partitioned between pH 4 buffer and CH_2Cl_2, extracted with CH_2Cl_2, dried, and evaporated to afford the alcohol as a white foam. This material was subsequently dissolved in CH_2Cl_2 and treated with Et_3N (0.72 mmol, 0.10 mL) and acetic anhydride (0.5 mmol, 0.04 mL) at 5 °C. After 1 h, the reaction was quenched into pH 9 buffer and extracted with CH_2Cl_2, dried, and concentrated to afford crude acetate. Recrystallization from EtOAc afforded **11p** (131 mg, 60%): mp 191−192 °C; ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H), 3.01 (s, 6H), 3.76 (s, 2H), 6.86 (s, 1H), 7.33 (t, J = 8 Hz, 1H), 7.44 (t, J = 7 Hz, 1H), 7.49−7.63 (m, 3H), 7.66−7.80 (m, 3H), 7.85 (s, 1H), 7.97 (d, J = 8 Hz, 1H), 8.05 (s, 1H), 8.13 (s, 1H); MS (DCI, isobutane) m/z (rel intensity) 460 (100) [-HOAc], 520 (40.5); Anal. ($C_{29}H_{24}F_3N_3O_3$), C,H,N.

***N,N*-Dimethyl-3-(9-oxo-9H-fluoren-2-yl)-1-[3-(trifluoromethyl)phenyl]-1H-pyrazole-4-acetamide (11r).** $KMnO_4$ (89.2 mmol, 14.1 g) was dissolved in 400 mL of water and added to a basic solution of nBuNSO$_4$ (4.1 mmol, 1.42 g) in KOH (26 mmol, 1.67 g in 210 mL of water). This mixture was added to **11n** (43 mmol, 20 g) in CH_2Cl_2 (400 mL), and the mixture was stirred overnight at RT. Solid KH_2PO_4 was added until the reaction was neutral, and then solid $NaHSO_3$ was added until the MnO_2 was reduced. The organic layer was separated and extracted with CH_2Cl_2, dried, and concentrated to an oil that was chromatographed over silica (EtOAc) to afford **11r** (16 g, 78%): mp 144−146 °C; ^{1}H NMR ($CDCl_3$) δ 3.03 (s, 6H), 3.76 (s, 2H), 7.29−7.37 (dt; J = 7,1 Hz, 2H), 7.48−7.72 (m, 6H), 7.84−7.93 (m, 1H), 7.96 (d, J = 7 Hz, 1H), 8.05 (s, 1H), 8.12 (s, 1H); MS (DCI, isobutane) m/z (rel intensity) 476 (100). Anal. ($C_{27}H_{20}F_3N_3O_2$), C,H,N,F. A second material was also obtained (3 g, 12%) that corresponded to an additional oxidation at the methylene of the acetamide side chain to afford a ketoamide.

3-(9-Hydroxy-9-methyl-9H-fluoren-2-yl)-*N,N*-dimethyl-1-[3-(trifluoromethyl)phenyl]-1H-pyrazole-4-acetamide (11q). To **11r** (3.4 mmol, 1.62 g) in THF (50 mL) at −60 °C was added MeMgCl (3.6 mmol, 1.2 mL in THF). The reaction was stirred for 30 min and then allowed to warm to RT. Workup entailed quenching into aq. NaH_2PO_4 and extracting with MTBE, drying, and evaporating to a crude oil. Chromatography over silica (EtOAc to 10% ethanol/EtOAc) afforded **11q** as a solid (1.41 g, 84%): mp 170−172 °C; ^{1}H NMR ($CDCl_3$) δ 1.80 (s, 3H), 1.84 (s, 1H), 2.99 (s, 6H), 3.76 (s, 2H), 7.31−7.44 (dp J = 7.5,1 Hz, 2H), 7.50−7.74 (m, 6H), 7.87 (s, 1H), 7.96 (d, J = 8 Hz, 1H), 8.06 (s, 1H), 8.11 (s, 1H); MS (DCI, isobutane) m/z (rel intensity) 474 (100) [-H_2O], 492 (33.6); Anal. ($C_{28}H_{24}F_3N_3O_2$), C,H,N,F.

Acknowledgment. The authors thank Dr. Michael J. Shapiro and Bertha Owens for NMR and MS analysis. We also thank Dr. Edwin Villhauer for helpful comments while reviewing this manuscript.

Supporting Information Available: Additional spectral and analytical data for analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cowie, C. C., Eberhardt, M. S. *Diabetes 1996 Vital Statistics*; American Diabetes Association: Alexandria, VA.
(2) Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-germ complications in insulin-dependent diabetes mellitus. *New Eng. J. Med.* **1993**, *329*, 977−986.
(3) Sowers, J. R.; Standley, P. R.; Ram, J. L.; Jacober, S.; Simpson, L.; Rose, K. Hypinsulinemia, Insulin Resistance, and Hyperglycemia: Contributing Factors in the Pathogenesis of Hypertension and Atherosclerosis. *Am. J. Hypertens.* **1993**, *6*, 260S−270S.
(4) Depres, J. P.; Lamarche, B.; Mauriege, P.; Cantin, B.; Dagenais, G. R.; Moorjani, S.; Lupien, P. J. Hyperinsulinemia as an Independent Risk Factor for Ischemic Heart Disease. *New Engl. J. Med.* **1996**, *334*, 952−957.
(5) Nestler, J. E.; Clore, J. N.; Blackard, W. G. Dehydroepiandrosterone: The "Missing Link" Between Hyperinsulinemia and Atherosclerosis? *FASEB J.* **1992**, *6*, 3073−3075.
(6) (a) King, A. B. A Comparison in a clinical setting of the efficacy and side effects of three thiazolidinediones *Diabetes Care* **2000**, *23*, 557. (b) Sood, V.; Colleran, K.; Burge, M. R. Thiazolidinediones: A Comparitive review of approved uses. *Diabetes Technol. Ther.* **2000**, *2*, 429−440.
(7) Zhang, B. B.; Moller, D. E., New Approaches in the Treatment of Type 2 Diabetes. *Curr. Opin. Chem. Biol.* **2000**, *4*, 461−467.
(8) **1** increased glucose transport into 3T3-L1 cells by 159% of control (@100 μM), in adipocytes by 131% of control (@ 300 μM) and in L6 muscle cells by 146% of control (@300 μM).
(9) **1** was tested in the following receptor binding assays: alpha 1, alpha 2, D1, D2, and opiate with $PIC_{50} < 6$.
(10) Irwin, S. Comprehensive obervational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacologia* **1968**, *13*, 222−257.
(11) Sarges, R.; Hank R. F.; Blake, J. F.; Bordner, J.; Bussolotti, D. L.; Hargrove, D. M.; Treadway, J. L.; Gibbs, E. M. Glucose Transport-Enhancing and Hypoglycemic Activity of 2-Methyl-2-phenoxy-3-phenylpropanoic Acids. *J. Med. Chem.* **1996**, *39*, 4783−4803.
(12) Wright, J. B.; Dulin, W. E.; Markillie, J. H. The Antidiabetic Activity of 3,5-Dimethylpyrazoles. *J. Org. Chem.* **1964**, *7*, 102−105.
(13) (a) Soliman, R.; Faid-Allah, H. M.; El Sadany, S. K. Preparation and Antidiabetic Activity of New Substituted 3,5-Diarylpyrazolesulfonylurea Derivatives II: Structure−Activity Relationship. *J. Pharm. Sci.* **1987**, *76*, 626−632. (b) Masataka, F.; Shimago, K.; Okano, S.; Nakatani, H.; Hesaka, K. Esters of 3-Pyrazolemethanols and Process for their Preparations. 1972 United States Patent # 3,666,756.
(14) Kees, K. L.; Fitzgerald, J. J. Jr.; Steiner, K. E.; Mattes, J. F.; Mihan, B.; tosi, T.; Mondoro, D.; McCaleb, M. L. New Potent Antihyperglyceimc Agents in d*b*/d*b* Mice: Synthesis and Structure−Activity Relationship Studies of (4-substitutedbenzyl)-(trifluoromethyl)pyrazoles and -pyrazolones. *J. Med. Chem.* **1996**, *39*, 3920−3928.
(15) (a) Rainer, G. Pyrazol-4-Acetic Acid Compounds. Byk Gulden Lomberg Chemische Fabrik GmbH. 1979 U.S. Patent # 4,146,-721. (b) Newberry, R. A. Pyrazole Compounds 1970 London Patent # 1373212.
(16) Various NSAIDs were evaluated in L6 myocytes for their potential effects on glucose transport. The following effects on glucose uptake were observed in L6myocytes with compound @ 300 μM and represented as % of control: acetominophen 83%; ibuprofen 68%; indomethacin 110%; lonazolac 81%; **1** 146%; insulin (1μM) 161%; for a description of the L6 myocytes assay see Aicher, T. D. et al. *J. Med. Chem.* **1998**, *41*, 4556.
(17) Kira, M. A.; Abdel-Rahman, M. O.; Gadalla, K. Z. The Vilsmeier−Haack Reaction III Cyclization of Hydrazones to Pyrazoles. *Tetrahedron Lett.* **1969**, 109−110.
(18) (a) For a review of the various transformations of the Willgerodt reaction see Brown, E. V. The Willgerodt Reaction *Synthesis* **1975**, 358−375. (b) For discussion of mechanisms see (1) Maier, L. Helv. Chim. Acta **1970**, *53*, 1216 or (2) Carmack, M. the Willgerodt−Kindler Reactions. 7. The Mechanisms *J. Heterocycl. Chem.* **1989**, *26*, 1319−1323
(19) Carmack, M.; Davis, C. The Willgerodt Reaction. V. Substituted Acetamides from β-substituted Acrylic Acids. *J. Org. Chem.* **1947**, *12*, 76−78.
(20) Generally behavioral effects were only noted with compounds where Ar2 was unsubstituted aromatic as in **1**. Therefore, the pyrrolidine amide of **1** also demonstrated marked behavioral effects. With Ar2 other than unsubstituted phenyl no other substitutions at either R1 or R3 had any behavioral-like effects except for the phenol ester of **8.** This activity was not observed in any other compound from Table 3 including the acid itself (**8**).
(21) Only tested up to 100 mg/kg.
(22) Roberts, R. M. G. Conformational Analysis of Biphenyls using ^{13}C NMR Spectroscopy. *Magn. Reson. Chem.* **1985**, *23*, 52−54.
(23) Bolton, R.; Burley, R. E. M. Stability of Carbonium Ions. Part 3. The Transmission of Substituent Effects Across the Fluorene and Biphenyl Systems. *J. Chem. Soc., Perkin Trans. 2* **1977**, *4*, 426−429.
(24) (a) Duncia, J. V.; Santella, J. B. III;, Chiu, A. T.; Wong, P. C.; Wexler, R. R. Dibenzobiclyclo [2.2.2]octane Angiotensin II Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **1995**, 5, 214−246. (b) Burnelli, S.; Varioli, L.; Guarnieri, A.; Lumachi, B. Nonsteroidal Antiinflammatories. XXII. Chiral Arylhydroxyfluorenylpropionic acids. *Bull. Chim. Farm.* **1991**, *130*, 133−135. (c) Grunewald, G. L.; Carter, A. E.; Sall, D. J.; Monn, J. A. Conformational Preference for the Binding of Biaryl Substrates and Inhibitors to the Active Site of Phenylehtanolamine *N*-Methyltransferase. *J. Med. Chem.* **1988**, *31*, 60−65.

(25) Values for **11v**/**11w** cLogP: 6.4/6.4; caco-2 penetration ($P_{app} \times 10^{-5}$) 1.1/2.2; microsomes (stability) (rat-$T_{1/2}$ − min) stable/18.2.
(26) cLogP values were determined by the method of Biobyte using CLOGP3. The traditional shake-flask method for determining LogP values was not possible for this series of compounds due to their insolubility in water. As these compounds are not ionizable, LogD would be of no additional value.
(27) Caco-2 penetration ($P_{app} \times 10^{-5}$) 1.7/2.1 − Apparent permeabilities (P_{app}) across caco-2 cells apical to basolateral at 120 min.
(28) The dosed compound was dissolved in a 40% microemulsion vehicle in water. Microemulsion vehicle: 43% Cremophor RH40, 35.75% corn oil-monoglyceride, 10.62% propylene glycol, and 10.62% ethanol. Vehicle itself had no significant effects on metabolic parameters in ob/ob mice.
(29) A comparison of **11g** and **11n**, which have similar cLogP values (5.9 and 5.8, respectively) and are both insoluble in water (<0.04 mg/mL, pH 6.8) yet had a 10-fold difference in ethanol solubility (24 and 2 mg/mL, respectively), illustrates the problems associated with formulating these compounds as drug substances
(30) cLogP values were determined by the method of Biobyte using CLOGP3. A plot of cLogP versus activity in vivo (ob/ob mice) provided no correlation (data not shown).
(31) (a) Tontonoz, P.; Hu, E.; Spiegelman, B. M. Stimulation of Adipogenesis in Fibroblasts by PPARγ2, a Lipid-activated Transcription Factor. *Cell* **1994**, 79, 1147−1156. (b) Hu, E.; Tontonoz, P.; Spiegelman, B. M. Transdifferentiation of Myoblasts by the Adipogenic Transcription Factors PPARγ and C/EBPα. *Proc. Nat. Acad. Sci. U.S.A.* **1995**, *92*, 9856−9580.
(32) (a) Lehman, J. M.; Moore, L. B.; Smith-Oliver, T. A.; Wilkinson, W. O.; Willson, T. M.; Kliewer, S. A. An Antidiabetic Thiazolidinedione is a High Affinity Ligand for Peroxisome Proliferator-Activated Receptor γ (PPAR-γ) *J. Biol. Chem*. **1995**, *270*, 12953−12956. (b) Willson, T. M.; Cobb, J. E.; Cowan, D. J.; Wiethe, R. W.; Correa, I. D.; Prakash, S. R.; Beck, K. D., Moore, L. B., Kliewer, S. A., Lehmann, J. M. The Structure Activity Relationship Between Peroxisome Proliferator-Activated Receptor γ Agonism and the Antihyperglycemic Activity of Thiazolidinediones. *J. Med. Chem*. **1996**, *39*, 665−668.
(33) (a) Yoshioka, S.; Nishino, H.; Shiraka, T.; Ikeda, K.; Koike, H.; Okuno, A.; Wada, M.; Fujiware, T.; Horikoshi, H. Antihypertensive Effects of CS-045 Treatment in Obese Zucker Rats. *Metabolism* **1993**, *42*, 75−80. (b) Buchanan, T. A.; Meehan, W. P.; Jeng, Y. Y.; Yang, D.; Chan, T. M.; Nadler, J. L.; Scott, S.; Rude, R. K.; Hsueh, W. A. Blood Pressure Lowering by Pioglitazone: Evidence for a Direct Vascular Effect. *J. Clin. Invest*. **1995**, *96*, 354−360. (c) DeSouza, C. J.; Yu, J. H.; Robinson, D. D.; Ulrich, R. G.; Meglasson, M. D. Insulin Secretory Defect in Zucker fa/fa rats is Improved by Ameliorating Insulin Resistance. *Diabetes* **1995**, *44*, 984−991. (d) Sugiyama, Y.; Taketomi, S.; Shimura, Y.; Ikeda, H.; Fujita, T. Effects of Pioglitazone in Glucose and Lipid Metabolism in Wistar Fatty Rats. *Arzmeim-Forsch*. **1990**, *40*, 263−267.
(34) Mori, Y., Murakawa, Y., Okada, K., Horikoshi, K., Yokoyama, J., Tajima, N., Ikeda, Y.; Effects of Troglitazone on Body Fat Distribution in Type 2 Diabetic Patients. *Diabetes Care* **1999**, *22*, 908−912.
(35) Compounds were evaluated as to 2-deoxyglucose uptake and phosphorylation in fully differentiated 3T3-L1 adipocytes (as per Experimental Section) in the presence or absence of 1 nM submax insulin. Cells were treated with compounds for 18 h in serum-free DMEM, washed and incubated for 30 min in the presence or absence of 1 nM insulin, followed by 2-DG uptake measurement. Both **11g** and troglitazone increased 2-DG uptake by 200−300% over control values @ 100 μM.
(36) Hepatocytes were isolated from the livers of 18 h fasted Sprague−Dawley rats (weighing 280−290 g) by the method of Berry and Friend. (Berry, M. N., Friend, D. S. High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structure study. *J. Cell Biol*. **1969**; *43*, 506−520.) Cells were incubated in the presence or absence of compounds at the indicated concentrations for 30 min at 37 °C before the addition of 10 mM lactate, 1 mM pyruvate, and 0.5 mM [1-^{14}C]oleate. The specific activity of [1-^{14}C]oleate (Du Pont-NEN, Boston, MA) was 1 mCi/mmol. After 30 min additional incubation at 37 °C, aliquots of the cell suspensions were immediately placed in an ice bath and were deproteinized with an equal volume of ice-cold 12% trichloroacetic acid (TCA). Supernatants from the centrifuged hepatocyte suspensions were assayed for glucose content with a spectroscopic (A_{505}) automated glucose oxidase method and for acid-soluble products (~95% ketone bodies in control incubations) from the ^{14}C radioactivity via liquid scintillation counting. Total cellular ATP concentrations were determined enzymatically from the TCA supernatants with phosphoglycerate phosphokinase and glyceraldehyde phosphate dehydrogenase (Sigma kit 366-UV). **11g** had no effect up to 300 μM. Troglitazone (@300 μM) exhibited effects on fatty acid oxidation (increased 25%) and glucose production (decreased 60%), and ATP levels were reduced to 25% of control.
(37) Sufficient plasma was taken at 4 h to obtain both glucose and insulin levels. Day 3-4 h insulin levels were 820 μU/mL ($p < 0.05$) vs 1995 μU/mL vehicle controls.
(38) Procedure same as for glucose lowering in ob/ob mouse described in experimental study except duration for 7 days and $n = 10$ per group. Control animals gained weight over the time period (47 g, day 1; 51 g, day 7). On day 7, body weights were significantly decreased as compared to control in the metformin (350 mg/kg/day: 48 g, +1 g, $p < 0.05$) and **11g** (15 mg/kg/day: 47 g, no change, $p < 0.05$) groups but were unchanged compared to controls in the troglitazone (200 mg/kg/day: 51 g, +4 g) treated animals. Food intake was reduced modestly by 10% for **11g** as compared to troglitazone and vehicle matched control.
(39) Carrageenan oedema model: Compounds were administered orally in a vehicle of Tween 80/tragacanth 1 h prior to carrageenan. 0.1 mL of 1% carrageenin suspended in physiological saline was given by sub-plantar injection into a hind paw. The control reading was taken immediately after the injection (0-h value), and the swelling was measured after 3 h by means of an antiphlogometer. Four rats (Tuttlingen, 150−170 g) were used per group. The mean values of the 3-h readings after deduction of the corresponding 0-h readings were calculated. Results are presented as % inhibition of paw swelling in treated animals versus controls. Results: diclofenac (3 mg/kg) 79%; lonazolac (5 mg/kg) 47%; **11g** (5 mg/kg) 36%.
(40) Study performed as per normal 3 day dosing of ob/ob mice as explained in Experimental Section except study was run for 7 days. Efficacy values @4 h postdose were day 1, 37.8%; day 2, 45.8%; day 3, 71.1%; and day 7, 74.4% (All values were statistically significant ($p < 0.05$)
(41) These results are from in-house investigations run parallel to lead compounds **11g** and **11n** in ob/ob mice under the normal protocol.
(42) These data are for the pyrrolidine amide. Pyrrolidine and dimethyl amides were found to be equivalent in activity in all other cases as indicated in text. The lack of activity of the dimethyl amide in this case is considered an aberration.

JM010032C