New Potent Antihyperglycemic Agents in db/db Mice: Synthesis and Structure−Activity Relationship Studies of (4-Substituted benzyl)(trifluoromethyl)pyrazoles and -pyrazolones

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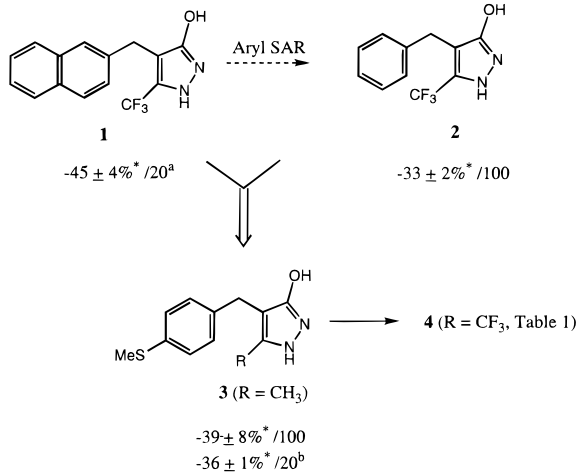
Received June 20, 1996[⊗]

The synthesis, structure−activity relationship (SAR) studies, and antidiabetic characterization of 1,2-dihydro-4-[[4-(methylthio)phenyl]methyl]-5-(trifluoromethyl)-3*H*-pyrazol-3-one (as the hydroxy tautomer; WAY-123783, **4**) are described. Substitution of 4-methylthio, methylsulfinyl, or ethyl to a benzyl group at C_4, in combination with trifluoromethyl at C_5 of pyrazol-3-one, generated potent antihyperglycemic agents in obese, diabetic db/db mice (16−30% reduction in plasma glucose at 2 mg/kg). The antihyperglycemic effect was associated with a robust glucosuria (>8 g/dL) observed in nondiabetic mice. Chemical trapping of four of the seven possible tautomeric forms of the heterocycle by mono- and dialkylation at the acidic hydrogens provided several additional potent analogs (39−43% reduction at 5 mg/kg) of the lead **4** as well as a dialkylated pair of regioisomers that showed separation of the associated glucosuric effect produced by all of the active analogs in normal mice. Further pharmacological characterization of the lead WAY-123783 (ED_{50} = 9.85 mg/kg, po in db/db mice), in oral and subcutaneous glucose tolerance tests, indicated that unlike the renal and intestinal glucose absorption inhibitor phlorizin, pyrazolone **4** does not effectively block intestinal glucose absorption. SAR and additional pharmacological data reported herein suggest that WAY-123783 represents a new class of potent antihyperglycemic agents which correct hyperglycemia by selective inhibition of renal tubular glucose reabsorption.

Recently, we described the discovery of 5-alkyl-4-(arylmethyl)pyrazol-3-ones (hydroxy tautomers) as potential new oral antidiabetic agents, based on their ability to lower plasma glucose when administered orally to obese, diabetic db/db mice.[1] A wide variety of 2-naphthyl and benzo heterocycles were found to possess oral activity at C_4 of the heterocycle, while the alkyl substituent at C_5 was limited to lower alkyl groups and amino. However, the best activity found belonged to the simple naphthalene analog **1** (Chart 1). During the course of structure−activity relationship (SAR) investigations on the aryl component of these structures, it was also found that phenyl analog **2** showed statistically significant activity, provided trifluoromethyl was at C_5.[1] Because the potency of the bicyclic arenes appeared to reach a plateau (20−40% decrease in plasma glucose) at a dose level of ~20 mg/kg/per day × 4, we sought to increase potency[2] by exploring the effects of additional substituents on the phenyl ring.

Initially, a 4-methylthio group was added to the phenyl ring since sulfur requires about the same volume element as the annulated (2nd) ring comprising 2-naphthyl and the hetero rings of the benzo heterocycles.[3] In vivo in the db/db mouse this approximation was born out as 4-[[4-(methylthio)phenyl]methyl]-5-methylpyrazolone (**3**) (Chart 1) showed comparable activity to **1** without a trifluoromethyl group at C_5. But pyrazolone **3** did not normalize the plasma glucose at high doses (Chart 1), nor did **3** maintain glucose lowering for 18−24 h after the last dose as desired. These features were attained when the trifluoromethyl group was placed at C_5, generating a new lead structure, pyrazolone **4** (Table 1).

Chart 1. Genesis of Potent Antihyperglycemic Pyrazolones



[a] Figures are percent mean ± SE change in plasma glucose of db/db mice (n = 6−10) at the dose (mg/kg/day × 4) indicated. [b] Mean of two experiments. *$p \leq 0.05$ for all data.

In this paper, we report the structure−activity relationship studies in db/db mice that identified a potent series of glucose-lowering analogs of **4**, including the separation of an associated glucosuric effect produced by these compounds in normoglycemic mice. In addition, results from ancillary pharmacologic profiling of

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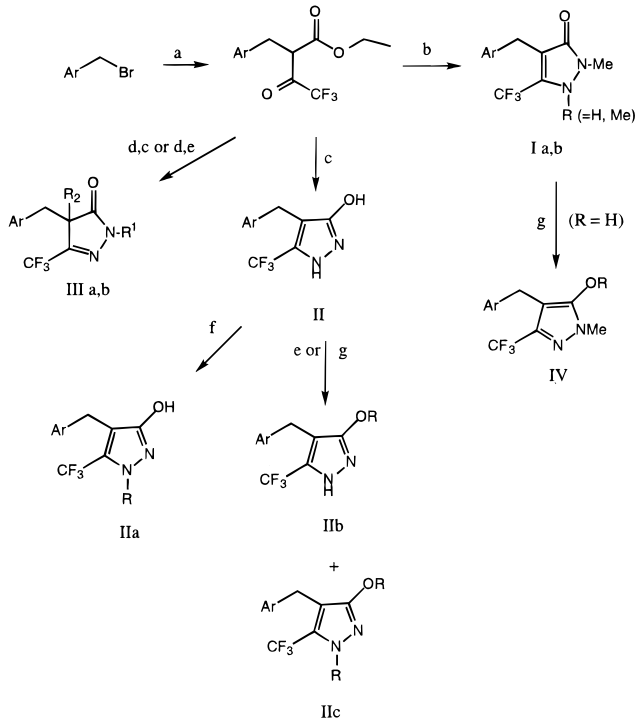
[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1996.

S0022-2623(96)00444-X CCC: $12.00

Scheme 1. Synthetic Routes to 3*H*-Pyrazol-3-ones and "Trapped" Tautomers **I**−**IV**[a]



[a] (a) Ethyl 4,4,4-trifluoroacetoacetate NaH, DME, reflux; (b) MeNHNHR·2HCl, Et(*i*-Pr)$_2$N, 3 Å molecular sieves, toluene reflux; (c) anhydrous hydrazine, toluene or DME, ±powdered 3 Å molecular sieves, reflux; (d) MeI, NaH, DME, room temperature; (e) MeI, K_2CO_3, CH_3CN, room temperature; (f) BuLi (2 equiv), THF, −78 °C, then MeI (1 equiv) → room temperature; (g) MeI, K_2CO_3, CH_3CN, reflux.

4 (WAY-123783) in several rodent models of non-insulin-dependent diabetes and in normal and insulin-dependent diabetic rats are reported which indicate that **4** and its analogs represent a new class of antihyperglycemic agents that correct hyperglycemia by selective inhibition of renal tubular glucose reabsorption.

Chemistry

The majority of the compounds, 3*H*-pyrazol-3-ones (**1**−**4**, **7**−**20**, **23**−**27**, Tables 1−3), were prepared by a two-step sequence starting from 4-substituted benzyl bromides as shown in Scheme 1 (Sequences a, c). Reaction of ethyl 4,4,4-trifluoroacetoacetate with NaH in anhydrous DME at ice temperature followed by addition of the benzyl halide and reflux provided the corresponding β-keto esters, which were treated with hydrazine to give the trifluoromethyl-substituted hydroxy pyrazoles **II** (Scheme 1). Aprotic conditions were employed throughout to avoid hemiketal formation at the trifluoromethyl ketone center.[4]

The benzyl halides which were not commercially available were routinely prepared by reduction of carboxylic acids/derivatives to carbinols followed by bromination with HBr, BBr_3, or CBr_4/Ph_3P. Alternatively, the corresponding 4-substituted tolyl compounds were brominated with NBS in refluxing carbon tetrachloride (e.g., **7, 8, 20, 25**). Quenching of 4-tolylmagnesium chloride (4-chlorotoluene, Mg, aluminum triisopropoxide, THF) with trifluoromethyl disulfide gave 4-[(trifluoromethyl)thio]toluene and starting chloride (∼3:2, respectively (NMR)). This mixture was brominated (NBS, CCl_4, reflux) and carried on as described above to give a mixture of pyrazolones **7** and **25**, which were separated by reverse phase chromatography (RP-C_{18} silica gel, gradient 50:50 H_2O:MeOH → 90:10 elution). The starting material for isopropyl sulfide derivative **9** was prepared from 4-bromobenzyl alcohol by sequential treatment with *n*-BuLi (2 equiv, TMEDA, −78 °C) and diisopropyl disulfide.

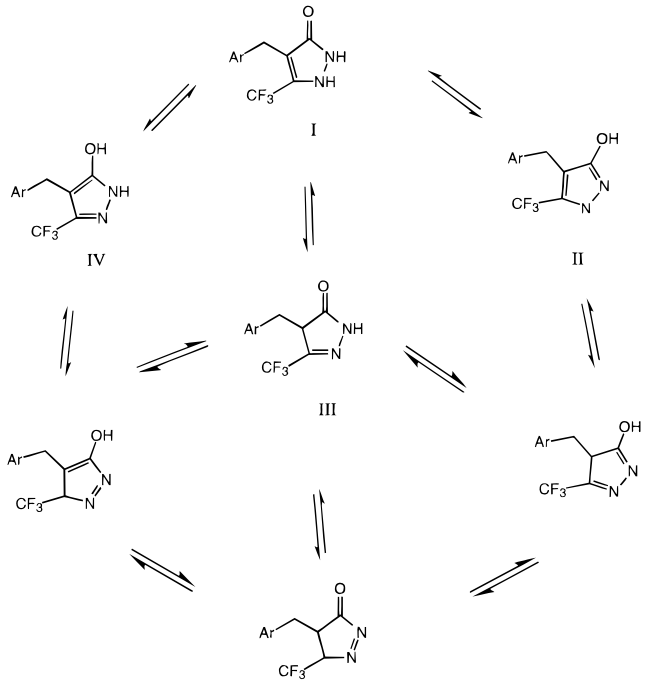
Oxidation of alkyl phenyl sulfide **4** with 30% hydrogen peroxide at room temperature gave the sulfoxide **5**, whereas oxidation of **4** with excess oxone gave the sulfone **6**. Exposure of 4-(4-acetylbenzyl)pyrazole **20**[5] to hydroxylamine (hydrochloride, pyridine, room temperature) gave oximes **21** (1:1 syn:anti). Reduction of **20** with $NaBH_4$ (EtOH, room temperature) gave carbinol **22**. 4-[4-(Ethylamino)benzyl]pyrazolone **28** was prepared as in Scheme 1 (a, c) starting from 4-(chloromethyl)phenylacetamide followed by reduction with diborane (THF, 0 °C).

Alkylation of hydroxypyrazoles of type **II** (Scheme 1) with 1 equiv of alkyl halides at room temperature in acetonitrile usually gave separable mixtures of N_1- and 3-O-alkylated products (**IIa**,**b**, Scheme 1) along with starting material. TLC monitoring of the reaction with methyl and ethyl iodides indicated that N_1-alkylation occurred first, but after 5−48 h *O*-alkyl is the major product along with N_1,3-O-dialkylated and N_1-alkylated (5-hydroxy) products in minor amounts. Using excess alkylating agent and/or refluxing acetonitrile conditions, a separable mixture of N_1,3-O- (**IIc**, Scheme 1) and N_1,5-O- (**IV**, Scheme 1) dialkylated products was obtained, the former predominating.[6] However, the N_1,5-O-dialkylated materials could be obtained in higher yield as the sole product of alkylation of 5-hydroxypyrazoles of type **IV** (R = H, Scheme 1, vide infra). In this way the mono- and dialkylation products **32**−**39** (Table 4) and **42** (Table 5) were prepared.

In the case of iodoethane alkylation of 4-[4-(methylthio)benzyl]-substituted hydroxypyrazole **4** under reflux conditions, the two main dialkylated products **38** and **42** were contaminated with inseparable *S*-ethyl impurities (see footnotes, Tables 4 and 5) via alkyl exchange.[7] In order to obtain greater quantities of minor alkylation products **31**, **40**, and **41**, alternative synthetic routes were employed.

Treatment of pyrazolone **4** with 2 equiv of *n*-BuLi (1 equiv of TMEDA, THF, −78 °C) and quenching of the dianion with 1 equiv of MeI gave N_1-methyl-5-hydroxypyrazole **31**. Reaction of ethyl 3-[4-(methylthio)phenyl]-α-(trifluoromethyl)propionate with methylhydrazine in refluxing toluene (b, Scheme 1) gave N_1-methylpyrazol-5-one **40**, as the aromatic enol tautomer (type **IV**, Schemes 1 and 2). None of the less polar (TLC, 70−30 hexane−EtOAc) isomer **31** was detected.[8] Assignments of the tautomeric forms of these structures were made on the basis of the solution cell ($CHCl_3$) infrared spectra, which indicated the presence of an OH stretching absorption and the complete absence of a C=O stretching band, and NOE experiments.[9] Confirmation of the regioisomeric assignments of **31** and the more polar isomer **40** were made on the basis of ^{13}C NMR. The chemical shift of the *N*-methyl group in **31** (δ 37.4, $CDCl_3$) is downfield from that of isomer **40** (δ 33.8, $CDCl_3$) and split into a quartet (J = 2.3 Hz) by long range coupling to the trifluoromethyl group, whereas the methyl group in **40** is a singlet.[7] Likewise, assign-

Scheme 2. Potential Tautomeric Forms of 4-(Arylmethyl)-5-(trifluoromethyl)pyrazol-3-ones



ments were made to the dialkylated isomeric pairs **36** (least polar isomer),**41** and **38**,**42** (more polar isomer).

N-Benzylpyrazolone **43** was prepared as in **40** using benzylhydrazine. Like **40**, compound **43** exists in solution as the enol tautomer (type **IV**, R = H, Scheme 1), and alkylation with methyl iodide occurred exclusively at oxygen to give **44**. Reaction of ethyl 2-[4-(methylthio)benzyl]-3-oxo-4,4,4-trifluorobutyrate with $N_{1,2}$-dimethyl hydrazine gave hydrated pyrazolone **45** (Table 5). NMR, IR, MS, and CHN data indicate this compound was isolated covalently bound to water, apparently via the Michael addition of water.[10]

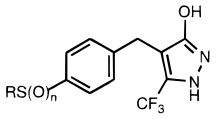
Alkylation of ethyl 2-[4-(methylthio)benzyl]-3-oxo-4,4,4-trifluorobutyrate (NaH, MeI, room temperature) followed by hydrazine treatment gave pyrazolone **46** (d, c, Scheme 1; IR ($CHCl_3$) ν 1740 cm^{-1}). Alkylation of **46** (e, Scheme 1) gave **47**.

Results and Discussion

SAR. Oxidation of the sulfide **4** to sulfoxide **5** (Table 1) surprisingly did not affect low-dose efficacy, whereas oxidation to the sulfone **6** destroyed activity. Additionally, none of the regioisomeric sulfides, sulfoxides, and sulfones showed significant glucose-lowering activity in the db/db mouse.[11] Substitution of trifluoromethyl for methyl on the sulfide moiety of **4** (**7**) significantly decreased activity, but increasing the alkyl chain length to ethyl (**8**) was tolerated. Addition of a branching methyl (isopropyl, **9**) or further chain lengthening to *n*-butyl (analog **10**) was deleterious. Provided that the sulfides, not sulfoxides, are the active species in vivo,[12] these results suggested that appropriate hydrocarbon units might function in the role of the alkyl sulfide group to yield potent analogs of **4** and avoid potential metabolic issues such as in vivo transformation of **4** to chiral **5**.

We thus prepared a number of hydrocarbon analogs of **4**. The results (**11**−**23**, Table 2) are consistant with those in the sulfide series. One hydrocarbon unit, ethyl

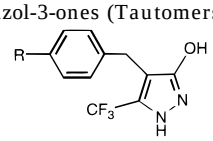
Table 1. SAR of 4-(4-Sulfur-substitutedbenzyl)-5-(trifluoromethyl)pyrazol-3-ones



no.	R	*n*	yield[a] (%)	mp (°C)[b]	dose (mg/kg)	db/db[c] change (%)
4	Me	0	20	147.5−148.5	100	−68 ± 2**
					20	−57 ± 1**[d]
					5	−43 ± 4**[e]
					2	−29 ± 2*[f]
					0.5	−13 ± 5
5	Me	1	29	200.5−201.5	5	−33 ± 2**
					2	−25 ± 2*
6	Me	2	56	223−225	5	2 ± 5
7	CF_3	0	6	125−127	20	−15 ± 6[e]
					5	5 ± 6
8	Et	0	7	133−134	20	−38 ± 2**
					5	−25 ± 3*
					2	−23 ± 3
9	*i*-Pr	0	9	169−170	20	−32 ± 5**
10	*n*-Bu	0	18	95−97.5	20	−3 ± 9

[a] Yields are for analytically pure material obtained in the last step and are not optimized. [b] Analyses (C,H,N) were within ±0.4% of theoretical values unless otherwise indicated. [c] Groups of db/db mice (n = 4−6) were administered either drug or vehicle (Tween 80/saline) once daily po × 4 days. Values (mean ± SE) are the percent change in plasma glucose concentration of drug-treated mice relative to vehicle controls at the given dose (mg/kg); NA = not active, generally less than −15% change; *p < 0.05 compared to vehicle control, **p < 0.01 compared to vehicle control. [d] Mean of three experiments. [e] Mean of two experiments. [f] Mean of six experiments.

Table 2. SAR of 4-(Carbon-substitutedbenzyl)-5-(trifluoromethyl)pyrazol-3-ones (Tautomers)

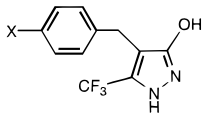


no.	R	yield[a] (%)	mp (°C)[b]	dose (mg/kg)	db/db[c] change (%)
11	Me	34	150−151.5	20	−36 ± 3*
12	Et	44	128−130	5	−39 ± 4**
				2	−28 ± 12**
				0.5	−11 ± 3
13	*n*-Pr	54	114−116[d]	20	−44 ± 3**
14	*i*-Pr	13	139−141	5	−17 ± 3
15	*n*-Bu	9	105.5−108	5	−7 ± 5
16	*t*-Bu	27	194.5−196	20	−7 ± 4[e]
17	*n*-hexyl	25	102−103[f]	20	5 ± 4
18	CF_3	56	123−124.5	5	−15 ± 6**
19	$(CF_3)_2CF$	25	170−172	20	1 ± 5
20	acetyl	6	218.5−219.5	5	−19 ± 7**
21	$CH_3C{=}NOH$	74	194−196	5	−28 ± 5**
22	CH_3CHOH	64	154−155	5	−18 ± 6
23	$(CH_3)_3CCO$	88	204−206	100	−23 ± 4

[a−c] See Table 1 footnotes. [d] Analysis for $0.25H_2O$. [e] Mean of two experiments. [f] Analysis for $0.9H_2O$.

(**12**), stands out as clearly the most potent analog, comparable to **4**, while shorter (**11**) and longer (**13**, **15**, **17**) or branched (**14**, **16**) chains markedly decreased activity, but perfluorocarbon substitution offered no dramatic effects (compare **11** vs **18**, **14** vs **19**). With the optimum 2-carbon chain length, polar groups which are capable of being either hydrogen bond donors or acceptors were evaluated (**20**−**23**, Table 2). Acetyl analog **20** and its oxime derivative **21** were efficacious at 5 mg/kg, while carbinol **22** and tertiary aryl ketone **23** showed no significant activity at 5 and 100 mg/kg, respectively.

Table 3. SAR of 4-(Heteroatom-substitutedbenzyl)-5-(trifluoromethyl)pyrazol-3-ones (Tautomers)



no.	X	yield[a] (%)	mp (°C)[b]	dose (mg/kg)	db/db[c] change (%)
24	F	8	159−160	20	−20 ± 5
				5	−3 ± 2
25	Cl	28	151−152	5	−6 ± 7
26	Br	42	135−136.5	20	−47 ± 3**
				5	−24 ± 7**
				2	−5 ± 9
27	I	45	132−134	20	−48 ± 2**[d]
28	EtN	40	169−171[e]	120	−61 ± 4 **
				5	−11 ± 3*[d]
29	MeO	45	181−183	5	−22 ± 7**
30	CF_3O	46	90−91	5	−4 ± 5

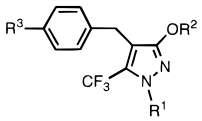
[a−c] *See Table 1 footnotes.* [d] Mean of two experiments. [e] Analysis for HCl(salt)·0.52-propanol·0.75H_2O. Compound sinters at 104 °C, liquifies over the range indicated.

With two distinct potent compounds in hand (**4**, **12**), we sought to further diversify the nature of the 4-benzyl substituent by examining the effects of various halogen and heteroalkyls and -aryls. A summary of some of our findings are presented in Table 3. In the halogen series (**24**−**27**, Table 3), only the larger halogen atoms bromine (**26**) and iodine (**27**) retained efficacy, but at best these analogs appear to belong to a group of second-tier compounds in potency compared to **4** and **12**. Likewise active analogs containing other heteroatoms were limited to short alkyl chains, but none of these analogs could match **4** and **12** in both efficacy and potency. For example, ethylamino analog **28** normalizes glucose at a high dose but loses potency at 5 mg/kg (Table 3). Similarly, methoxy analog **29** retains low-dose efficacy but appears to belong to the second-tier group of less potent compounds than **4** and **12**. Trifluoromethoxy **30** abolished low-dose activity, a result comparable to the sulfide pair **4**,**7** but in contrast to the alkyl pair **11**,**18** (compare also **24** vs **2**[1]). It appears from the SAR results in Tables 1−3 that steric effects are the more dominant factor controlling activity rather than electronic factors.

Continuing our search for additional potent compounds, working back toward the heterocycle in lead **4**, we found that the methylene linker between the 4-substituted phenyl and pyrazolone rings was critical for activity,[11] and so we returned our attention to the heterocyclic ring. As addressed previously,[1] these 4-benzyl-5-(trifluoromethyl)pyrazol-3-ones exist exclusively as the aromatic hydroxy tautomer in solution. Since, in principle, seven tautomeric forms are possible (Scheme 2), we sought to trap these tautomeric forms by alkylation at the acidic hydrogens. Using a variety of synthetic methods (Scheme 1), several sets of mono- and dialkylated materials were obtained (see the Chemistry section), corresponding to four of the seven tautomers (see **I**−**IV**, Schemes 1 and 2).

In the series of monomethylated pyrazolones **31**−**33** (Table 4), **40**, and **46** (Table 5), *O*-methyl analog **32** (and most likely *O*-methyl **33**) retains potency and efficacy comparable to **4** and **12**. In contrast both N_1-methyl-3-hydroxypyrazole **31** and the N_1-methyl-5-hydroxy isomer **40** are inactive at 5 mg/kg. The monomethyl analog at C_4 (**46**) did not show significant activity at 20 mg/kg dose, nor did N_1-benzyl **43** (Table 5). *O*-Isopropyl and *O*-*n*-butyl analogs **34** and **35** (Table 4) retained significant, but decreased, activity compared to **4** at 20 mg/kg.

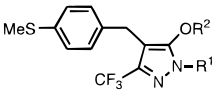
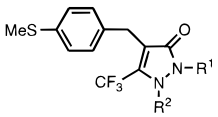
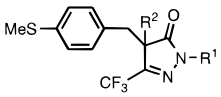
Table 4. SAR of N_1- and 3-O-Alkylated (Trifluoromethyl)pyrazoles and -pyrazol-3-ones (Tautomers)



no.	R^1	R^2	R^3	yield[a] (%)	mp (°C)[b]	dose (mg/kg)	db/db[c] change (%)
31	Me	H	MeS	18	141.5−145.5	20	−29 ± 6**
						5	−7 ± 2[d]
32	H	Me	MeS	12	115−117	20	−57 ± 3**
						2	−16 ± 6*
33	H	Me	Et	27	108−109[e]	5	−36 ± 4**
34	H	*i*-Pr	MeS	36	92−93.5	20	−40 ± 9**
35	H	*n*-Bu	Et	46	oil	20	−34 ± 3**
36	Me	Me	MeS	36	31−34	20	−49 ± 3**
						5	−41 ± 5**
						2	−20 ± 5
37	Me	Me	Et	40	oil	5	−38 ± 0**
38	Et	Et	MeS	38	oil[f]	5	5 ± 8
39	*n*-Bu	*n*-Bu	Et	17	oil[g]	20	−3 ± 4

[a−c] See Table 1 footnotes. [d] Mean of two experiments. [e] Analysis for 1H_2O. [f] Inseparable mixture containing ∼25% SEt (NMR). [g] Analysis for 0.5H_2O.

Table 5. SAR of N_1,5-O, $N_{1,2}$-, and N_1,C_4-Alkylated (trifluoromethyl)pyrazoles and -pyrazol-5-ones

no.	R^1	R^2	yield[a] (%)	mp (°C)[b]	dose (mg/kg)	db/db[c] change (%)
						
40	Me	H	38	138−139		−40 ± 4**
					5	−11 ± 3
41	Me	Me	70	oil	5	−39 ± 3**
42	Et	Et	10	oil[d]	5	−23 ± 3*
43	$C_6H_5CH_2$	H	16	169−170	20	10 ± 4
44	$C_6H_5CH_2$	Me	15	oil		NT
						
45	Me	Me	12	glass[e]	20	−6 ± 5
						
46	H	Me	30	117−118	20	−9 ± 2
47	Me	Me	31	oil	20	6 ± 5

[a−c] See Table 1 footnotes. [d] Inseparable mixture (∼94:6) of MeS and EtS (NMR). [e] Analysis for 2H_2O. C: calcd, 50.30; found, 50.80. IR, NMR, and MS suggest 1 mol of covalently bound water (Micheal addition product).[10]

From the results in the series of dimethylated pyrazolones **36**, **37** (Table 4), **41**, **45**, and **47** (Table 5), it is apparent either nitrogen can be methylated without loss in potency, provided the oxygen is also methylated (compare **36**, **37** vs **41**, **4**, **12**). In contrast, when oxygen is not alkylated, activity is abolished ($N_{1,2}$-dimethyl **45**, N_1,C_4-dimethyl **47**, Table 5). Homologs of the potent dimethylated compounds gave more dramatic effects. N_1,5-*O*-diethyl analog **42** (Table 5) shows significant glucose-lowering activity at 5 mg/kg dose, but the response is diminished relative to that of the corresponding dimethyl **41,** and the isomeric N_1,3-*O*-diethyl **38** showed no significant glucose-lowering activity at 5

mg/kg. As expected, N_1,3-*O*-di-*n*-butyl analog **39** was inactive at 20 mg/kg.

In summary, investigations into the SAR of mono- and dialkylated pyrazolone tautomers resulted in the identification of four additional potent antihyperglycemic agents: *O*-methyl derivative **32**, N_1,3-*O*-dimethyl analogs **36** and **37**, and N_1,5-*O*-dimethyl **41**, yielding a series (including **4** and **12**) of six potent glucose-lowering pyrazoles and pyrazolones for further pharmacological characterization.

Pharmacology Profile of WAY-123783 (4). As can be seen from dose−response data in Table 1, WAY-123783 produced a 68% reduction in plasma glucose in db/db mice at 100 mg/kg. It essentially normalized glucose levels at 20 mg/kg (57% reduction) and retained significant reduction at 2 mg/kg (∼30% decrease). However, no significant glucose reduction occurred at 0.5 mg/kg. The dose−response data indicate that WAY-123783 has an oral ED_{50} of 9.85 mg/kg. At a dose of 20 mg/kg, WAY-123783 normalized plasma glucose in db/db mice 4 h after the initial dose and maintained significant reduction for 24 h.[13] In another experiment to measure acute effects, streptozotocin-induced diabetic rats (a model of insulin deficiency) were fasted for 3 h prior to administration of WAY-123783 (25 mg/kg, po). WAY-123783 showed no significant reduction in plasma glucose during a period of 0.5−3 h after drug administration (data not shown). In no cases was frank hypoglycemia observed in these diabetic rodents.

In order to assess the effects of WAY-123783-induced plasma glucose reduction on plasma insulin levels, we administered the drug to obese, diabetic ob/ob mice. Unlike db/db mice, which have very high plasma glucose levels but normal or slightly elevated circulating plasma insulin levels at the age used in these studies,[1] ob/ob mice have only modest hyperglycemia but very high insulin levels. Both models represent severe insulin resistance and obesity and neither respond to the clinically efficacious insulin-releasing sulfonylurea agents.[14]

WAY-123783, at a dose of 20 mg/kg/day × 4, administered orally as a solution in 1% aqueous $NaHCO_3$, significantly decreased (−30%) plasma glucose in the ob/ob mouse (Figure 1A), comparable to ciglitazone[15] and phlorizin,[16] two known antihyperglycemic agents which do not release insulin. Similar to ciglitazone, the reduction in plasma glucose induced by WAY-123783 was accompanied by even greater reductions in plasma insulin concentrations (≥−50%, Figure 1B), which were not statistically significant due to variability in the control group.

During the course of continued pharmacological characterization of WAY-123783, we observed a robust glucosuric effect produced by the compound in normal mice.[17] Subsequent to this finding, virtually all of the compounds reported in this and previous work[1] were screened in the normal (Swiss CD) mouse for glucosuria at 100 mg/kg/day × 4. The results of this study can be summarized as follows. All compounds that showed significant plasma glucose reduction in the db/db mouse were positive in the glucosuria screen.[18] There did not appear to be a correlation between the magnitude of glucosuria induced in normal mice and potency or efficacy of the compounds as glucose-lowering agents in db/db mice. In addition, many acidic azoles prepared

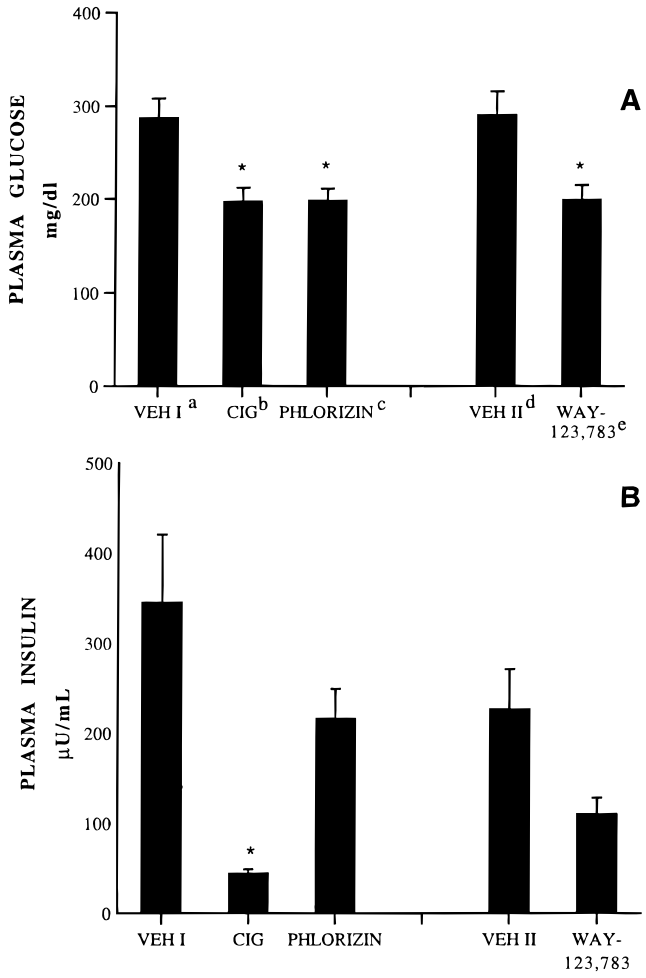


Figure 1. Effect of drugs on plasma glucose and insulin in ob/ob mice. [a]2% Tween 80/saline. [b]Ciglitazone (in vehicle I) administered po once a day for 4 days at 100 mg/kg. [c]Phlorizin (in vehicle I) administered ip once a day for 4 days at 200 mg/kg. [d]1% aqueous $NaHCO_3$ solution. [e]WAY-123783 (in vehicle II) administered po once a day for 4 days at 20 mg/kg. *p < 0.01.

by us and others[19] produced significant glucosuria in normal mice but were not active at high (e.g., 100 mg/kg) doses in the db/db mice. Consistent with these results, the SAR reported here produced several examples where the glucose-lowering effect in db/db mice could be titrated away (e.g., **10**, **17**, **19**, **39**, **43**) while a robust glucosuria in the normal mouse was maintained. One significant example of this trend concerned the pyrazole isomers **38** and **42**. Whereas compound **42** significantly lowered plasma glucose levels in db/db mice at 5 mg/kg, **38** showed no glucose-lowering effect at this dose, yet each produced approximately equivalent glucosuria in normal mice.[20] Our data suggest that if an association exists between glucosuria and plasma glucose lowering produced by these azoles, glucose lowering must occur secondarily to a marked increase in glucose excretion into the urine.

Because glucosuria in normal mice indicates altered renal function, possibly altered tubular reabsorption of glucose, it was of interest to compare the effects of WAY-123783 with phlorizin on glucose tolerance tests (GTTs) in normal and obese, insulin-resistant rats. Phlorizin has long been recognized as an inhibitor of the active sodium−glucose cotransporter (SGLT) present in both kidney and gut.[21] Inhibition of SGLT in the kidney

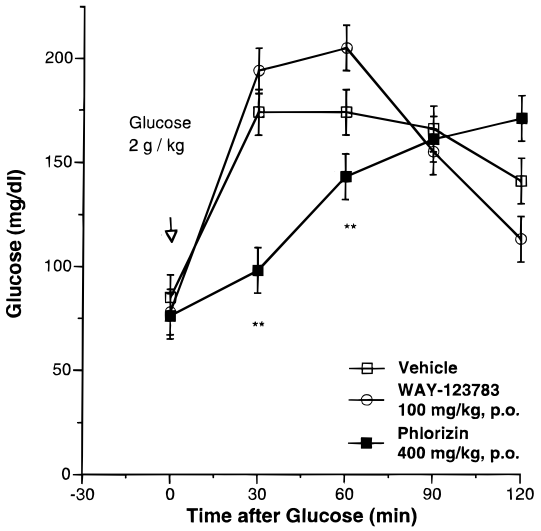


Figure 2. Drug effects on plasma glucose during an OGTT in normal rats. Rats were administered either vehicle (0.5% methylcellulose) or drug in the morning. One hour later, D-glucose was administered by oral gavage. Blood was collected from the tail tip at 0 (pre), 30, 60, 90, and 120 min after carbohydrate administration, and plasma glucose levels were determined by an Hibachi 911 analyzer. $^{**}p < 0.01$ (Dunnett's one-tailed *t*-test).

causes marked glucosuria due to inhibition of renal tubular reabsorption of filtered glucose (from urine), whereas inhibition of SGLT in the intestine blocks absorption of dietary glucose. The effects of phlorizin on intestinal glucose absorption can be measured by monitoring the appearance of glucose in the plasma following the administration of a glucose load.

Oral administration of glucose (2 g/kg) to 18 h fasted Sprague−Dawley (normal) rats caused a characteristic steep rise in plasma glucose concentration (Figure 2) that was significantly blunted by administration of phlorizin (400 mg/kg, po[22] 1 h prior to glucose challenge). In contrast, WAY-123783, at 100 mg/kg, did not block the sharp rise in plasma glucose following an oral glucose challenge (Figure 2).[23] These results indicate that WAY-123783 differs from phlorizin by its apparent incapacity to effectively block intestinal glucose absorption.[24]

The SAR data reported here support the notion that, like phlorizin, WAY-123783 and, presumably, its analogs are antihyperglycemic agents which act by blocking SGLT in the kidney. The data are thus consistent with WAY-123783 acting in a renal specific manner to block tubular glucose reabsorption. The recent discovery of SGLT2, an isoform of SGLT1 (intestinal Na^+−glucose cotransporter), as the principal mediator of renal glucose reabsorption,[21] provides an explanation for the observed differential effects of phlorizin and WAY-123783 on intestinal glucose absorption.

In summary, substitution of 4-methylthio, methylsulfinyl, or ethyl to a benzyl group at C_4, in combination with trifluoromethyl at C_5, of pyrazol-3-one (hydroxy tautomer) generated potent antihyperglycemic agents in obese, diabetic db/db mice (16−30% reduction in plasma glucose levels at 2 mg/kg dose). Chemical "trapping" of four of the seven possible tautomeric forms of the heterocycle by mono- and dialkylation at the acidic hydrogens provided several additional potent analogs (39−43% reduction at 5 mg/kg) of the methylthio lead pyrazolone **4** (WAY-123783), including *O*-methyl analog **32**, N_1,3-*O*-dimethyl analogs **36** and **37**, and N_1,5-*O*-dimethylpyrazole **41**.

Pharmacological characterization of the lead WAY-123783 revealed a robust glucosuric effect produced by the compound when administered to normal mice. Subsequent evaluation of all of the analogs indicated that plasma glucose lowering in diabetic animals is likely a (secondary) consequence of glucosuria induction, but there did not appear to be a correlation between the magnitude of the glucosuria (qualitatively determined as 0.1−12 g/dL) produced in normal mice and the potency or efficacy of glucose lowering in db/db mice. Oral glucose tolerance tests in normal, 18 h fasted rats with a single, high dose of WAY-123783 (100 mg/kg) administered 1 h prior to glucose challenge indicated that, unlike phlorizin, the pyrazolone does not appear to block intestinal glucose absorption. Our data suggest that WAY-123783 represents a new class of low-dose effective antihyperglycemic agents which correct hyperglycemia by selective inhibition of renal glucose reabsorption. The recent identification of SGLT2, a low-affinitity, high-K_m Na^+−glucose contransporter responsible for ∼90% of filtered glucose reabsorption in the early proximal tubule, provides a possible molecular mechanism for the observed effects of the compound on basal circulating glucose levels in diabetic rodents and the changes in plasma glucose following administration of an oral glucose load.

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-200, Varian VXR-300, or Bruker AM-400 instrument. IR spectra were recorded on a Perkin-Elmer diffraction grating or Perkin-Elmer 784 spectrophotometer. Mass spectra were recorded on a LKB-9000S, Kratos MS 50, or Finnigan 8230 mass spectrometer. Elemental analyses were obtained with a Perkin-Elmer 2400 elemental analyzer, and all compounds were within 0.4% of theoretical values unless otherwise noted. All reactions were carried out under inert atmosphere (N_2 or Ar). HPLC purifications were carried out on a Waters Prep 500 or Prep 500A instrument. "Standard aqueous workup" involves separation of an organic phase, washing it with saturated aqueous NaCl solution, drying over anhydrous $MgSO_4$, filtration, and concentration in vacuo on a rotary evaporator.

1,2-Dihydro-4-[[4-(methylthio)phenyl]methyl]-5-(trifluoromethyl)-3*H*-pyrazol-3-one (Tautomer, 4). A solution of 4-(methylthio)benzyl alcohol (20 g, 0.13 mol) and carbon tetrabromide (47.4 g, 0.14 mol) in dichloromethane (450 mL) was cooled to 0 °C under N_2 atmosphere. Triphenylphosphine (37.4 g, 0.14 mol) was added portionwise over 0.5 h and the resulting mixture allowed to warm gradually to ambient temperature. The reaction mixture was poured onto saturated aqueous NaCl solution. Following standard aqueous workup, the residue was filtered through a silica gel column with the aid of dichloromethane. Volatile materials were removed on the rotary evaporator, and the residue was triturated with hot heptane. The heptane solution was allowed to stand at room temperature overnight, filtered, and concentrated. This process was repeated twice with petroleum ether to give a quantitative yield of [4-(methylthio)phenyl]methyl bromide as a yellow mobile oil.

Sodium hydride (2.76 g, 69 mmol, 60% oil dispersion) in 1,2-dimethoxyethane (50 mL) was cooled to 0 °C. Ethyl 4,4,4-trifluoroacetoacetate (12.7 g, 69 mmol) was added dropwise at a rate so as to control H_2 evolution. When the addition was complete the homogeneous solution was warmed to reflux temperature and a solution of [4-(methylthio)phenyl]methyl

bromide (15 g, 69 mmol) in 1,2-dimethoxyethane (100 mL) was added. The reaction mixture was refluxed 15 h and cooled to room temperature, and volatile materials were removed in vacuo on the rotary evaporator. The residue was partitioned between 1 N aqueous HCl solution and ethyl acetate followed by standard aqueous workup. The residue was Kugelrohr distilled at reduced pressure (~0.5 mm Hg). Excess starting β-keto ester was collected below 100 °C oven temperature, and the desired ethyl 2-[4-(methylthio)benzyl]-3-oxo-4,4,4-trifluorobutyrate (12.2 g, 38 mmol, 55%) was collected at 115−140 °C oven temperature.

A mixture of ethyl 2-[4-(methylthio)benzyl]-3-oxo-4,4,4-trifluorobutyrate (6.0 g, 19 mmol), anhydrous hydrazine (0.92 mL, 29 mmol), and toluene was refluxed for 15 h. The reaction mixture was cooled to ambient temperature and concentrated to a tan solid. The residue was triturated with hot toluene (steam bath). The solution was decanted, and the product crystallized on standing. The title compound, as white crystals, weighed 1.08 g (3.8 mmol) after 15 h in an Abderhalden apparatus (ethanol, reflux): IR (KBr) υ (cm^{-1}) 3230, 1600, 1540, 1515, 1490; ^1H NMR (400 MHz, DMSO-d_6) δ 2.41 (s, 3H), 3.68 (s, 2H), 7.03 (d, 2H, J = 8.2 Hz), 7.15 (d, 2H, J = 8.2 Hz), 10.79 (s, br, 1H), 12.84 (s, br, 1H); MS (EI) m/z 288 (M^+).

1,2-Dihydro-4-[[4-(methylsulfinyl)phenyl]methyl]-5-(trifluoromethyl)-3*H*-pyrazol-3-one (Tautomer, 5). Compound **4** (2.5 g, 8.7 mmol) in acetone (40 mL) was treated with aqueous hydrogen peroxide solution (1 mL, 30% solution, 9 mmol) at room temperature for 24 h. The precipitate was collected on a Buchner funnel, washed with H_2O and diethyl ether, and dried under vacuum to give 1 g (3 mmol) of white solid: IR (KBr) υ (cm^{-1}) 2860 (br), 1585, 1540, 1510, 1395, 1290, 1280, 1230, 1165, 1125, 1110, 1075, 1010, 995, 975, 950, 935, 895, 805, 770; ^1H NMR (400 MHz, DMSO-d_6) δ 2.68 (s, 3H), 3.8 (s, 2H), 7.28 (d, 2H, J = 8 Hz), 7.56 (d, 2H, J = 8 Hz), 10.88 (s, br, 1H), 12.89 (s, br, 1H); MS (EI) m/z 304 (M^+).

1,2-Dihydro-4-[[4-(methylsulfonyl)phenyl]methyl]-5-(trifluoromethyl)-3*H*-pyrazol-3-one (Tautomer, 6). To a solution of **4** (2.50 g, 8.68 mmol) in acetic acid (20 mL), ethanol (20 mL), water (20 mL), and concentrated sulfuric acid (10 mL) was added potassium peroxymonosulfate (oxone; 5.29 g, 8.6 mmol) in one portion. The mixture was stirred at room temperature for 15 h, another 2.6 g (4.3 mmol) of oxone was added, and the reaction mixture was stirred for an additional 24 h at room temperature. The reaction mixture was filtered and the filtrate was subjected to standard aqueous workup. The product, homogeneous by TLC (60−40 hexane−ethyl acetate + 1% acetic acid), was dissolved in a minimum amount of ethyl acetate (hot, steam bath), diluted with hexane until turbid, and then cooled in ice. The title compound, a white solid, was collected by vacuum filtration and air-dried: IR (KBr) υ (cm^{-1}) 3280, 3025, 2930, 2670, 1600, 1560, 1525, 1485, 1430, 1410, 1395, 1330, 1290, 1255, 1185, 1150, 1115, 1090, 1010, 1000, 985, 980, 910, 820, 790, 765, 690, 650; ^1H NMR (400 MHz, DMSO-d_6) δ 3.16 (s, 3H), 3.84 (s, 2H), 7.34 (d, 2H, J = 8.4 Hz), 7.81 (d, 2H, J = 8.4 Hz), 10.87 (br, 1H); MS (EI) m/z 320 (M^+).

1,2-Dihydro-4-[[4-(isopropylthio)phenyl]methyl]-5-(trifluoromethyl)-3*H*-pyrazol-3-one (Tautomer, 9). To a −78 °C solution of 4-bromobenzyl alcohol (25.0 g, 0.134 mol) in tetrahydrofuran (350 mL) and tetramethylethylenediamine (42.5 mL, 0.281 mol) was added a solution of *n*-butyllithium in hexane (202 mL, 1.6 M, 0.323 mol). The reaction mixture was held at −78 °C for 1.5 h, warmed to 0 °C for 0.5 h, and then recooled to −78 °C and a solution of diisopropyl disulfide (20 g) in tetrahydrofuran (300 mL) added dropwise. The mixture was allowed to warm to room temperature gradually and stirred for 15 h. The reaction mixture was cooled in ice, and the reaction was quenched with aqueous 1 N HCl solution followed by standard aqueous workup. The crude amber oil was partially purified by distillation on a Kugelrohr apparatus (undesired material collected at oven temperature 23−65 °C, ~0.5 mmHg). The pot residue contained 20.5 g (0.113 mol, 84%) of 4-(isopropylthio)benzyl alcohol which was used without further purification: IR (film) υ (cm^{-1}) 3350; ^1H NMR (200 MHz, $CDCl_3$) δ 1.28 (d, 6H), 2.6 (t, 1H), 3.35 (heptet, 2H), 4.68 (d, 2H); MS (EI) m/z 182 (M^+).

4-(Isopropylthio)benzyl bromide was prepared from the above alcohol (15.0 g, 82.3 mmol), carbon tetrabromide (32.8 g, 98.8 mmol), and triphenylphosphine (25.9 g, 98.8 mmol) as described for compound **4**, which provided 17.1 g (69.8 mmol, 85%) of the product as an amber semisolid: MS (EI) m/z 245 (M^+).

A solution of the bromide (15.0 g, 61.1 mmol) in DME was added to a warm solution of β-keto ester anion (from 9.7 mL, 66.2 mmol, of ethyl 4,4,4-trifluoroacetoacetate and 2.60 g, 66.2 mmol, of NaH, 60% oil dispersion) in DME, as described for compound **4**, and gave 8.5 g (24.4 mmol, 40%) of the [4-(isopropylthio)benzyl]acetoacetate, as an amber oil: IR (film) υ (cm^{-1}) 1770, 1745; ^1H NMR (200 MHz, $CDCl_3$) δ 3.25 (d, 2H), overlapping with outer lines of heptet at 3.3; MS (EI) m/z 348 (M^+).

A mixture of the above acetoacetate (8.5 g, 24.4 mmol), anhydrous hydrazine (1 mL, 31.3 mmol), and powdered 3A sieves (4.5 g) was heated in toluene (475 mL) at reflux overnight. The reaction mixture was filtered hot and concentrated in vacuo on a rotary evaporator and the residue partitioned between dichloromethane and 5 N HCl solution followed by standard aqueous workup. The residue was treated with hot benzene (steam bath) and filtered while hot, and the product deposited as white crystals upon cooling to room temperature, to give 0.695 g (2.2 mmol) of **9**: ^1H NMR (400 MHz, DMSO-d_6) δ 1.18 (d, 6H, J = 6.6 Hz), 3.37 (m, 2H, overlapping with H_2O peak), 3.7 (s, br, 2H), 7.05 (d, 2H, J = 8 Hz), 7.26 (d, 2H, J = 8 Hz); MS (EI) m/z 316 (M^+).

1,2-Dihydro-1-methyl-4-[4-(methylthio)phenyl]-5-(trifluoromethyl)-3*H*-pyrazol-3-one (Tautomer, 31). A mixture of compound **4** (2.50 g, 8.68 mmol), tetramethylethylenediamine (1.44 mL, 9.5 mmol, freshly distilled from calcium hydride), and tetrahydrofuran (30 mL) was cooled to −78 °C under N_2 atmosphere. A solution of *n*-butyllithium (9.10 mL, 18.2 mmol, 2 M in pentane) was added dropwise to the vigorously stirred reaction mixture. The mixture was held at −78 °C for 0.3 h, warmed to ice temperature for 0.25 h, and cooled to −78 °C, and methyl iodide (0.8 mL) was added dropwise. The reaction mixture was allowed to warm to ambient temperature, stirred for 15 h, and cooled in ice, and the reaction was quenched with 1 N aqueous HCl solution. Following standard aqueous workup, the residue (a thick black oil) was dissolved in a minimum amount of warm ethyl acetate and left to stand at room temperature for 13 days. Upon partial evaporation of solvent during the period, the title compound, as pale violet crystals, grew out of the sludge. The crystals were collected, washed with diethyl ether, and air-dried to give 0.472 g, 1.56 mmol, of **31**: IR ($CHCl_3$) υ (cm^{-1}) 3050, 2980, 1580, 1530, 1480, 1425, 1270, 1165, 1120, 1080, 1020, 1000, 955; ^1H NMR (400 MHz, $CDCl_3$) δ 2.45 (s, 3H), 3.78 (s, 5H, overlapping N-Me and CH_2; note: with 15 drops of C_6D_6 added, NMe shifts to 3.53, CH_2 @ 3.76), 7.16 (ABq, 4H, J = 8.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2, 26.8, 37.5 (q J = 2.3 Hz), 106.2 (q, J = 1.5 Hz), 120.2 (q, J = 270.4 Hz), 127.1, 128.6, 129.4 (q, J = 37.4 Hz), 135.8, 137, 159.4; MS (EI) m/z 302 (M^+).

4-[(4-Ethylphenyl)methyl]-3-methoxy-5-(trifluoromethyl)-1*H*-pyrazole (33). A mixture of compound **12** (2 g), anhydrous potassium carbonate (1.44 g, pulverized), and acetonitrile (25 mL) was refluxed for 1 h and cooled to room temperature, and methyl iodide was added to the stirred mixture in portions (4 × 0.44 mL) over 2 days. The reaction mixture was diluted with enough water to dissolve all salts and the mixture partitioned between ethyl acetate and saturated brine solution. The extracts were washed with saturated brine, dried over $MgSO_4$, and concentrated and the residue chromatographed on silica gel (35 wt equiv). Elution with 10% ethyl acetate/hexane provided the title compound as a white solid: IR (KBr) υ (cm^{-1}) 3240, (br OH/H_2O), 3020, 2980, 2920, 1510, 1470, 1450, 1420, 1405, 1315, 1240, 1225, 1165, 1120, 1025, 965, 935, 900, 835, 820, 775, 755, 720, 700, 660, 630; ^1H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, 3H, J = 7.6 Hz), 2.6 (q, 2H, J = 7.6 Hz), 3.78 (s, 2H), 3.93 (s, 3H), 7.11 (q, 4H, J = 8.2 Hz), 8.8−9.6 (1H); MS (EI) m/z 284 (M^+).

3-Ethoxy-1-ethyl-4-[[4-(methylthio)phenyl]methyl]-5-(trifluoromethyl)pyrazole (38) and 5-Ethoxy-1-ethyl-4-

[[4-(methylthio)phenyl]methyl]-3-(trifluoromethyl)pyrazole (42). A mixture of compound **4** (2.0 g, 6.9 mmol), anhydrous potassium carbonate (pulverized, 2.44 g, 17 mmol), and acetonitrile (25 mL) was refluxed for 1 h. Ethyl iodide (1.4 mL, 17 mmol) was added, and the mixture was refluxed overnight. The reaction mixture was cooled to room temperature and diluted with enough water to dissolve all salts followed by standard aqueous workup and chromatography on silica gel (35 wt equiv, elution with 10% ethyl acetate/hexane) to provide the title compound **38** (least polar isomer, 861 mg, 2.5 mmol) as a white solid. Further elution provided the more polar isomer **42** (237 mg, 0.69 mmol), as a yellow oil. (Note: both isomers were contaminated with inseparable SEt analogs as indicated in the tables.) **38:** IR (film) υ (cm^{-1}) 2980, 2930, 1580, 1510, 1490, 1475, 1405, 1390, 1360, 1305, 1210, 1175, 1115, 1095, 1045, 1015, 800, 765, 740; ^1H NMR (400 MHz, $CDCl_3$) δ 1.28* (t, 3H, J = 7.4 Hz), 1.36 (m (overlapping triplets), 6H), 1.55 (s, 3H), 2.89* (q, 2H, J = 7.4 Hz), 3.74 (s, br, 2H), 4.08 (q, J = 7.1 Hz), 4,23 (q, J = 7 Hz, 2H, 2H), 7.15 (m, 4H, overlapping with 2H*), 7.24* (d, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4*, 14.8, 15.5, 16.2, 26.8*, 28, 46 (partially resolved quartet), 64.7, 106.1, 120.6 (q, J = 270 Hz), 126.9, 127.9 (q, J = 37 Hz), 128.7, 129.5*, 133.6*, 135.5, 137.5, 138.3*, 160.1 (*absorptions assigned to SEt impurity); MS (EI) m/z 344 (M^+).

42: IR (film) υ (cm^{-1}) 2980, 2930, 1575, 1510, 1495. 1485, 1470, 1440, 1275, 1260, 1165, 1120, 1090, 1055, 1025, 980, 925, 890, 790. 720; ^1H NMR (400 MHz, $CDCl_3$) δ 1.27* (t, 3H, J = 7 Hz), 1.28 (t, 3H, J = 7.1 Hz), 1.42 (t, 3H, J = 7.1 Hz), 2.46 (s, 3H), 2.9* (q, 2H, J = 7 Hz), 3.82 (s, br, 2H), 3.93 (q, 2H, J = 7.1 Hz), 4.05 (q, 2H, J = 7.3 Hz), 7.08 (d, 2H, J = 8.4 Hz), 7.18 (d, 2H, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4*, 14.9, 15.4, 16, 27.4, 27.9*, 42.8, 70.8, 102.8, 121.6 (q, J = 269 Hz), 126.9, 128.4, 129.4*, 134*, 135.9, 136.8, 137.7*, 138.8 (q, J = 36 Hz), 151.3 (*absorptions assigned to SEt impurity); MS (EI) m/z 344 (M^+).

1-Methyl-4-[[4-(methylthio)phenyl]methyl]-3-(trifluoromethyl)-2*H*-pyrazol-5-one (Tautomer, 40). The title compound was prepared as in compound **4** synthesis except that methylhydrazine was used instead of hydrazine. Recrystallization from toluene−hexane mixture provided the title compound as off-white crystals: IR ($CHCl_3$) υ (cm^{-1}) 3100 (br), 1570, 1550, 1480, 1420, 1395, 1290, 1270, 1240, 1150, 1115, 1055; ^1H NMR (400 MHz, $CDCl_3$) δ 2.45 (s, 3H), 3.49 (s, 3H), 3.78 (s, 2H), 7.07 (d, J = 8.4 Hz), 7.17 (d, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9, 27.3, 33.8, 98.9, 121.4 (q, J = 269.7 Hz), 127.1, 128.7, 135.3, 136.8, 138 (q, J = 36.6 Hz), 150; MS (EI) m/z 302 (M^+).

5-Methoxy-1-methyl-4-[[4-(methylthio)phenyl]methyl]-3-(trifluoromethyl)pyrazole (41). A mixture of compound **40** (3.0 g, 9.9 mmol), anhydrous potassium carbonate (pulverized, 12 g, 87 mmol), and acetonitrile (275 mL) was refluxed for 2 h and cooled to room temperature, and methyl iodide (neat, 2.0 mL) was added. The reaction mixture was stirred at room temperature for 2.5 days. The mixture was filtered and concentrated in vacuo to provide analytically pure **41** (2.2 g, 6.9 mmol), as a mobile yellow oil: IR (KBr) υ (cm^{-1}) 3080, 3020, 2980, 2945, 2920, 1600, 1575, 1515, 1485, 1435, 1410, 1380, 1340, 1290, 1270, 1160, 1115, 1070, 1040, 1020, 990, 965, 950, 925, 825, 800, 775, 720, 715, 685; ^1H NMR (400 MHz, $CDCl_3$) δ 2.46 (s, 3H), 3.73 (s, 6H, overlapping N-Me and O-Me; in benzene-d_6, OMe @ 2.97, NMe @ 3.03), 3.84 (s, 2H), 7.09 (d, 2H, J = 8.4 Hz), 7.18 (d, 2H, J = 8.4 Hz); ^{13}C NMR (100 MHz, C_6D_6) δ 15.6, 27.5, 34.1, 60.9, 103, 122.8 (q, J = 269 Hz), 126.9−128.8 obscured by solvent), 136.9, 137.2, 138.9 (q, J = 36 Hz), 152.7; MS (+FAB) m/z 316 ($M + H)^+$.

1,4-Dimethyl-4-[[4-(methylthio)phenyl]methyl]-5-(trifluoromethyl)-2*H*-pyrazol-5-one (46). To a slurry of sodium hydride (60% oil dispersion, 1.37 g, 34.3 mmol) in anhydrous DME (300 mL) at −25 °C was added a solution of ethyl α-(trifluoroacetyl)-3-[4-(methylthio)phenyl]propionate (10.0 g, 31.2 mmol; see compound **4** synthesis) in DME (20 mL) at a rate so as to control H_2 evolution. When gas evolution ceased, the mixture was allowed to warm to ambient temperature and a solution of methyl iodide (5.50 g, 39.0 mmol) in DME (30 mL) was added dropwise. After 1.5 h at room temperature, the mixture was stirred at reflux for 15 h. The mixture was filtered, triturated with petroleum ether (hot, steam bath), concentrated in vacuo on the rotary evaporator, and dried under vacuum, to give 9.10 g (27.2 mmol, 87%) of ethyl 2-[4-(methylthio)benzyl]-3-oxo-4,4,4-trifluorobutyrate, as a mobile yellow oil: IR (film) υ (cm^{-1}) 1735; ^1H NMR (400 MHz, $CDCl_3$) δ 1.39 (s, 3H), 2.46 (s, 3H), 3.31 (dd, J_{ab} = 14 Hz); MS (EI) m/z 334 (M^+).

The title compound was prepared from the above β-keto ester (8.60 g, 25.7 mmol), anhydrous hydrazine (1.63 mL, 51.4 mmol), and 3 Å molecular sieves (powdered, 4.0 g) in toluene (350 mL) as for compound **4**. The reaction mixture was filtered hot and concentrated on the rotary evaporator and the residue crystallized from toluene to give (after drying on an abderhalden apparatus, refluxing acetone, 20 h) 2.30 g (7.62 mmol) of **46**, as yellow crystals: IR ($CHCl_3$) υ (cm^{-1}) 3430, 3220 (broad), 3020, 2990, 2820, 1740, 1600, 1490, 1450, 1425, 1405, 1395, 1385, 1185, 1140, 1060, 1035, 1010, 835, 800, 720; ^1H NMR (400 MHz, $CDCl_3$) δ 1.5 (s, 3H), 2.44 (s, 3H), 3.10 (ABq, 2H, J = 13.8 Hz), 7.04 (d, 2H, J = 8.4 Hz), 7.09 (d, 2H, J = 8.4 Hz), 8.6 (s, br, 1H).

1,4-Dimethyl-4-[[4-(methylthio)phenyl]methyl]-5-(trifluoromethyl)-2*H*-pyrazol-5-one (47). A mixture of **46** (3.0 g, 9.9 mmol), anhydrous potassium carbonate (pulverized, 12 g, 87 mmol), and acetonitrile (200 mL) was refluxed for 1 h. Methyl iodide (15.4 mL, 248 mmol) was added; the mixture was refluxed for 15 h followed by addition of methyl iodide (15.4 mL) and continued reflux for 24 h. The reaction mixture was cooled to ambient temperature, filtered, and concentrated. The crude product was passed through a short column of silica gel with the aid of hexane to give the title compound (960 mg, 3 mmol) as an amber syrup: IR (film) υ (cm^{-1}) 2975, 2920, 1725, 1600, 1585, 1490, 1450, 1435, 1420, 1400, 1380, 1345, 1310, 1230, 1170, 1135, 1120, 1100, 1035, 1020, 1010, 960, 930, 920, 830, 800, 755, 735, 710, 690; ^1H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 3H), 2.43 (s, 3H), 3.08 (s, 2H), 3.12 (s, 3H), 7.0 (d, 2H, J = 8.3 Hz), 7.09 (d, 2H, J = 8.4 Hz); MS (CI) m/z 317 (M + H)$^+$.

Postprandial ob/ob Mouse Assay. On the morning of day 1 (baseline), 56 ob/ob mice (C57Bl/6J, Jackson Laboratories, 3−4 months of age, body weight of 43−61 g) were randomly assigned into seven groups (n = 8) of equivalent mean body weight. Drug or vehicle (0.2 mL) was administered once daily for 3 days to the ad libitum fed mice. On the morning of day 4, food was removed from all cages, drug or vehicle was administered, and mice were fasted for the remainder of the experiment. Four hours later, mice were anesthetized with fluothane (halothane) and then quickly decapitated for blood collection into fluoride-containing tubes. Tubes were mixed and maintained on ice until centrifuged. Plasma was separated, and the levels of glucose in the plasma were determined by an Abbott VP analyzer. The remainder of the plasma was frozen until insulin could be determined by RIA, using the double-antibody technique.

Acknowledgment. We thank Dr. Mike Malamas for helpful suggestions, Bruce Hoffmann and staff for analytical services, Kathryn Santilli (NOE experiments), Marilyn Winkler, and Phyllis Totaro (manuscript preparation).

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(2) ED_{25} values, the effective oral dose for 25% reduction in plasma glucose, have dropped from 40 mg/kg (ciglitazone) to 0.05 mg/kg for some newer analogs; see: (a) Sohda, T.; Mizuno, K.; Mamose, Y.; Ikeda, H.; Fujita, T.; Meguro, K. Studies on Antidiabetic Agents. 11. Novel Thiazolidinedione Derivatives as Potent Hypoglycemic and Hypolipidemic Agents. *J. Med. Chem.* **1992**, *35*, 2617−2626. (b) Another series of potent thiazolidene-

diones in ob/ob mice have been recently described; see: Hulin, B.; Clark, D. A.; Goldstein, S. W.; McDermott, R. E.; Dambek, P. J.; Kappeler, W. H.; Lamphere, C. H.; Lewis, D. M.; Rizzi, J. P. Novel Thiazolidene-2,4-diones as Potent Euglycemic Agents. *J. Med. Chem.* **1992**, *35*, 1853−1864.

(3) van der Waals radii for sulfur and half-thickness of a phenyl ring are 1.85 Å; see: Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 3rd ed.; Interscience: New York, 1972; p 120.

(4) Hemiketal formation at the trifluoromethyl ketone carbonyl was facile in protic media, and this material was very slow to cyclize with hydrazine.

(5) Compound **20** was prepared by starting from commercially available 4-acetyltoluene and protection as the ethylene ketal followed by NBS, steps a and b (Scheme 1), and aqueous HC1.

(6) Sodium hydroxide in methanol or ethanol gave similar results.

(7) IR and ^{1}H and ^{13}C NMR data are given in the Experimental Section.

(8) A minor impurity in **40** was detected in the ^{13}C NMR spectrum, but the chemical shift (δ 38.8 (s), $CDC1_3$) indicates it is not **31**.

(9) Irradiation of the hydroxyl proton of **40** at δ 11.0 (DMSO-d_6) produced an NOE at the *N*-methyl (δ 3.61), benzylmethylene (δ 3.7), and the two adjacent ortho hydrogens (δ 7.0).

(10) IR (KBr) ν (cm^{-1}) 3240 (br), 2920, 1665 (very strong), 1495, 1165; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.46 (s, 3H), 2.59 (s, 3H), 3.02 (s, 3H), 3.13 (s, 3H), 3.28 (s, 1H); note: singlet at δ 3.28 exchangeswith D_2O; the spectrum does not change upon addition of 3 drops of C_6D_6, but sample in C_6D_6 alone shows an ABX pattern (δ 2.9− 3.1), 3−3 proton singlets at higher field, and a 1 proton singlet at δ 3.6; MS (DCI) *m*/*z* 335 ($M^+ + H$).

(11) M. L. McCaleb, unpublished results.

(12) We have no metabolic data concerning the possible in vivo interconversion of **4** and **5**.

(13) Four hours after administration of **4** (20 mg/kg), mean plasma glucose levels in db/db mice were decreased 59%; 24 h later, plasma glucose was decreased 22% vs controls.

(14) In our hands, neither first-generation (tolbutamide) nor second-generation (glyburide) sulfonylurea agents lowered plasma glucose in fed ob/ob or db/db mice. In db/db mice, glyburide (10 mg/kg/day × 4) did not significantly increase plasma insulin levels.

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(17) All drugs were administered to normal (Swiss CD) mice at 100 mg/kg, po/day × 4 (n = 3). On day 4, mice were placed into Nalgene metabolic cages (each group of three in a single cage) with ad libitum access to food and water. Urine was collected for 20−24 h, and urine glucose levels were qualitatively determined using an Abbot VP autoanalyzer.

(18) Urine glucose above 0.1 g/dL was considered "positive" for glucosuria.

(19) M. Malamas, I. Gunawan, M. McCaleb, unpublished results. See: Malamas, M. Naphthalenylmethyl Thiophenones as Antihyperglycemic Agents. U.S. Patent 5,444,086, 1995.

(20) Glucosuria: **38**, 7.65 g/dL; **42**, 7.3 g/dL.

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(22) Administration of phlorizin at 200 mg/kg, ip gave similar results.

(23) Nor did WAY-123783 block absorption of a subcutaneous infusion of glucose (1 g/kg) given to obese, insulin-resistant Zucker (fa/fa) rats, after 4 days of drug administration (25 mg/kg/day × 4); on the morning of the fourth day, Zucker rats (n = 4) were fasted for 2 h followed by the fourth administration of WAY-123783. After an additional 1 h fast, glucose (1 g/kg) was administered by subcutaneous infusion. Blood samples were collected from the tail tip at 0 (pre-glucose), 30, 60, 90, and 120 min after glucose. The area under the glucose curve was compared to that of vehicle-treated rats.

(24) In one experiment, WAY-123783 administered (100 mg/kg) simultaneously with glucose (2 g/kg, po) to 18 h fasted normal rats significantly inhibited glucose absorption 30 min postdose (∼15% inhibition).

JM960444Z