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Original article

Antihyperglycemic activity of new 1,2,4-oxadiazolidine-3,5-diones

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Abstract – A series of 1,2,4-oxadiazolidine-3,5-diones was synthesized and evaluated as oral antihyperglycemic agents in the obese insulin resistant db/db and ob/ob mouse — the two models for Type 2 diabetes mellitus. The majority of the prepared methoxy- and ethoxy-linked oxazole 1,2,4-oxadiazolidine-3,5-diones normalized plasma glucose levels at the 100 mg kg^{-1} oral dose in the db/db diabetic mouse model, and several amongst them reduced the glucose levels at the 20 mg kg^{-1} oral dose. The most potent compounds in the db/db mouse model were also active in the ob/ob mouse model normalizing the plasma glucose levels at the 20 mg kg^{-1} oral dose. The trifluoromethoxy analog **32** was the most active compound of the series, reducing significantly the plasma glucose levels at the 5 mg kg^{-1} oral dose. Oxadiazole-tailed 1,2,4-oxadiazolidine-3,5-diones were also active in both the db/db and ob/ob diabetic mouse models normalizing plasma glucose levels at the 100 mg kg^{-1} oral dose. © 2001 Éditions scientifiques et médicales Elsevier SAS

insulin resistance / antihyperglycemic agents / oxadiazolidinediones / diabetic animal models

1. Introduction

Insulin resistance in the liver and peripheral tissues, together with a pancreatic β-cell defect is the common cause of Type 2 diabetes [1]. The prevalence of insulin resistance in pre-diabetic or glucose intolerant subjects has long been recognized [2]. The failure of insulin to suppress adequately the hepatic glucose output postprandially, combined with the reduced glucose disposal by the peripheral tissues, leads to abnormal glycemic control after feeding. The increased and sustained plasma glucose levels progresses gradually into a number of debilitating diabetic complications as retinopathy, neuropathy, nephropathy, atherosclerosis, and coronary artery disease [3]. Therefore, it is essential to control blood glucose at the early stages of the disease.

Treatment of Type 2 diabetes consists usually of diet, exercise, and hypoglycemic agents. Sulfonylureas are the most widely used antidiabetic agents. These agents are acting on pancreatic β-cells stimulating insulin secretion. However, these agents are often known to induce severe hypoglycemia and weight gain, and become ineffective after 5 years of treatment [4–7]. Another group of drugs is the thiazolidinediones, which enhance insulin action. The PPARγ receptor, a member of the nuclear hormone receptor superfamily, has been identified recently as the major functional receptor for the thiazolidinediones [8]. Since the pioneering discovery of ciglitazone **1** (*figure 1*) by Takeda scientists [9], which reduced insulin resistance and normalized plasma glucose levels in genetically diabetic and/or obese animal models, a plethora of new thiazolidinediones has been developed [10–18]. Troglitazone **2**, a thiazolidinedione-type pharmacological agent, has been used effectively as an insulin enhancing agent in a large number of Type 2 patients [19][1]. However, troglita-

Abbreviations: PPARγ, peroxisome proliferator activated receptor γ.

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[1] Troglitazone is being marketed in the United States as Rezulin and in Japan as Noscal.

zone has produced severe liver toxic effects in a small number of patients, and has been withdrawn from the market. Recently, thiazolidinediones of SmithKline Beecham (rosiglitazone; **3a**) and Takeda (pioglitazone; **3b**) have been approved for marketing. Patients on these new agents will also be monitored for potential liver toxicity. Biguanides — a third class of oral-lowering agents — were associated with lactic acidosis, and only metformin (**3c**), offers a useful treatment for insulin-resistant overweight Type-2 diabetes patients [20, 21]. The glucose-lowering effect of metformin occurs without stimulation of insulin secretion and results mainly from increased glucose utilization. Metformin enhances insulin action at the postreceptor level in peripheral tissues such as muscle.

In the present study, we disclose a detailed and systematic structure–activity relationship (SAR) study of new 1,2,4-oxadiazolidine-3,5-diones as potent antihyperglycemic agents. Some limited information on oxadiazolidinediones as antihyperglycemic agents has been reported previously [15] in a series of oxazolylethoxy-hydroxyureas, which exhibited hypoglycemic properties.

2. Chemistry

The 1,2,4-oxadiazolidine-3,5-diones (*tables I–IV and VI*) were prepared according to *figure 2.* Treatment of either hydroxybenzaldehyde or hydroxyacetophenone with either oxa(thia)zoles **4** [22–24], oxadiazoles **5** [22, 25] or triazoles **6** [26] in the presence of potassium carbonate afforded azoles **7** ($n = 1$). Coupling of alcohols **8** with either hydroxybenzaldehyde or hydroxyacetophenone, using the Mitsunobu protocol [27], produced azoles **7** ($n = 2$). Azoles **7** were converted to the hydroxylamines **10** in a two-step process. First, azoles **7** were treated with hydroxylamine hydrochloride in the presence of sodium acetate to produce the corresponding oximes **9**, which were reduced further to the hydroxylamines **10** with sodium cyanoborohydride under acidic conditions. The hydroxylamines **10** were converted to the 1,2,4-oxadiazolidine-3,5-diones **11** with *N*-(chlorocarbonyl)isocyanate at low temperatures (0–5°C).

The benzoxazole **63** [28] (*table IV*) was prepared from 2-(2-benzoxazolylmethylamino)ethanol [17] and 4-hydroxybenzaldehyde substantially in the same manner as described above.

The benzofurans **68–72** (*table V*) were prepared according to *figure 3.* Condensation of oxazole **12** with 3-bromo-2-hydroxybenzaldehyde in the presence of sodium methoxide afforded benzofuran **13**. Reduction of the ketone functionality, first with sodium borohydride to the corresponding secondary alcohol, and second with triethylsilane/trifluoroacetic acid, produced benzofuran **14**. Conversion of the aromatic bromide of **14** to a formyl group (**16**) was achieved through a two-step process. First, the generation of the nitrile **15** with copper cyanide in *N*,*N*-dimethylformamide, and second, the reduction of **15** with Al–Ni in formic acid furnished **16**. Benzofuran **16** was converted to the final product **18** ($R^3 = H$) according to *figure 2.* Compound **18**, when $R^3 = CH_3$, was pre-

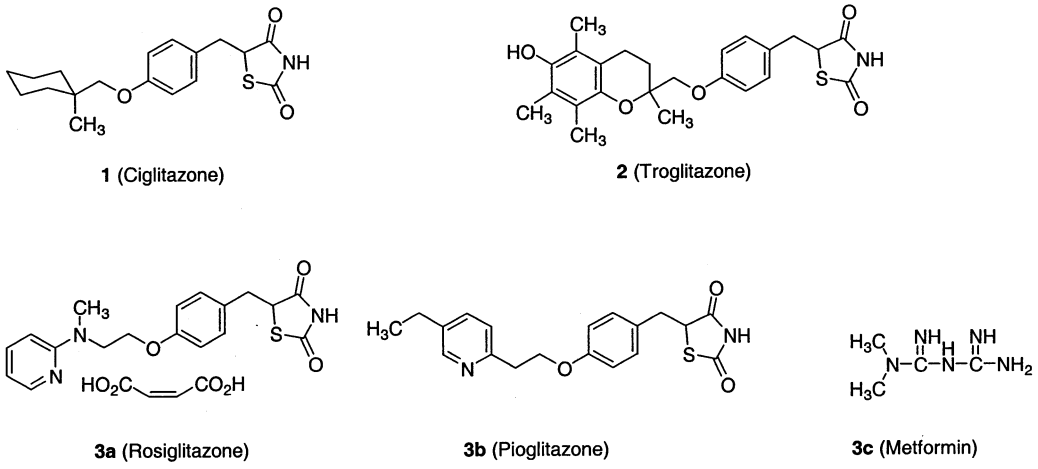
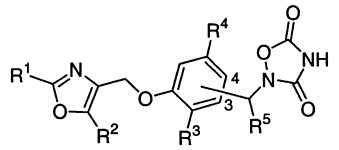


Figure 1. Known antihyperglycemic agents

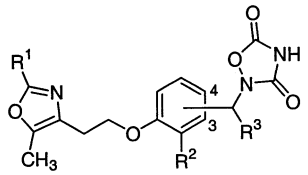
Table I. Chemical and biological data of methoxy-linked oxazole 1,2,4-oxadiazolidine-3,5-diones.[a]



Compound	R^1	R^2	R^3	R^4	R^5	POA [c]	m.p. (°C)	% Decrease in plasma glucose [b]			% Decrease in plasma insulin [b]
								db/db mouse 100 mg kg^{-1}	db/db mouse 20 mg kg^{-1}	ob/ob mouse 20 mg kg^{-1}	ob/ob mouse 20 mg kg^{-1}
19	Ph	CH_3	H	H	H	4	170–172	32*	*i* [d]		
20	Ph	CH_3	H	H	H	3	148–149	47*	*i*	*i*	66**
21	Ph	CH_3	H	H	CH_3	4	157–158	*i*			
22	Ph	CH_3	H	H	CH_3	3	159–160	*i*			
23	Ph	CH_3	F	H	H	4	184–185	16*			
24	Ph	CH_3	OCH_3	H	H	4	181–182	22*			
25	Ph	H	H	H	H	3	139–140	22			
26	4-CF_3–Ph	CH_3	H	H	H	4	174–175	37*			
27	4-CF_3–Ph	CH_3	H	H	H	3	142–143	60*	37*	21	44*
28	3-CF_3–Ph	CH_3	H	H	H	3	167–168	60*	17		
29	4-CF_3-Ph	CH_3	H	F	H	3	159–161	51*	24*		
30	4-CF_3-Ph	CH_3	H	OCH_3	H	3	168–170	42*	i		
31	3,5-CF_3, CF_3–Ph	CH_3	H	H	H	3	176–178	54*	i		
32	4-OCF_3–Ph	CH_3	H	H	H	3	162–163	64*	46*	47** 27* (5 mg kg^{-1}) 27* (100 mg kg^{-1})	68** 25* (5 mg kg^{-1}) 94* (100 mg kg^{-1})
33	3-OCF_3–Ph	CH_3	H	H	H	3	149–150				
34	4-F–Ph	CH_3	H	H	H	3	163–164	36*			
35	4-Cl-Ph	CH_3	H	H	H	3	166–167	37*			
Ciglitazone								32* [b]			
Troglitazone								34*			

[a] All compounds were prepared according to *figure 2.*
[b] All values for the drug-treated groups, other than *i*, are significant vs. vehicle-treated mice; * $P<0.05$; ** $P<0.01$.
[c] POA, Point of attachment to the phenyl ring.
[d] *i*, Inactive, generally less than −15% change, $P>0.05$.

Table II. Chemical and biological data of ethoxy-linked oxazole 1,2,4-oxadiazolidine-3,5-diones.[a]



Compound	R^1	R^2	R^3	POA [c]	m.p. (°C)	% Decrease in plasma glucose [b]	
						db/db mouse 100 mg kg^{-1}	db/db mouse 20 mg kg^{-1}
36	Ph	H	H	4	205–206	43*	45*
37	Ph	H	H	3	173–174	45*	*i* [d]
38	Ph	H	CH_3	4	169–170	40*	
39	Naphthyl	H	H	4	185–186	18*	*i*
40	4-OCH_3–Ph	H	H	4	201–202	30*	*i*
41	4-CH_3–Ph	H	H	4	196–197	*i*	
42	Cyclohexyl	H	H	4	130–132	57*	
43	Thienyl	H	H	4	205–206	*i*	
44	Furyl	H	H	4	200–202	43*	
45	4-CF_3–Ph	H	H	4	190–192	75*	
46	4-F–Ph	H	H	4	185–186	71*	
47	3-F–Ph	H	H	4	198–199	63*	
48	2-F–Ph	H	H	4	195–196	63*	
49	Ph	F	H	4	207–208	38*	

[a] All compounds were prepared according to *figure 2.*
[b] All values for the drug-treated groups, other than *i*, are significant vs. vehicle-treated mice, $P<0.05$.
[c] POA, Point of attachment to the phenyl ring.
[d] *i*, Inactive, generally less than -15% change, $P>0.05$.

pared similarly from methyl-ketone **17**. Ketone **17** ($R^3 = CH_3$) was generated during a two-step process from **16**. First, the formyl group was converted to the corresponding secondary alcohol with methylmagnesium chloride, and second, it was oxidized with Jones reagent to furnish **17**.

3. Results and discussion

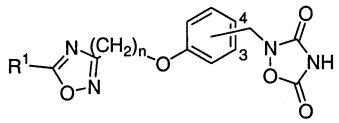
The test compounds were evaluated in two classical insulin-resistant hypoglycemic obese mouse models, the genetically obese C57 BL/6J ob/ob, and diabetic C57BL/KsJ db/db, for their ability to decrease plasma glucose and insulin levels. The ob/ob animal model is severely insulin resistant, hyperinsulinemic, and glucose intolerant, and the db/db model is also glucose intolerant with fasting hyperglycemia and occasional hyperinsulinemia. These animal models [29] have been validated well by several laboratories for the assessment of novel glucose lowering agents [10–18]. The *in vivo* activity was assessed at a daily dose of 5–100 mg kg^{-1} (p.o.) for 4 days, and measured as a specified decrease in plasma glucose and insulin levels of the drug-treated group relative to a vehicle-treated control group. Decreases of 50–60 (db/db) and 30–40% (ob/ob) in plasma glucose levels normalize generally the glucose levels that are similar to the non-diabetic controls. A compound considered active, at the specific dosage administered, if the difference of the plasma glucose level had a $P<0.05$. All compounds with $P>0.05$ were reported as inactive. Ciglitazone was the reference standard in all of the assays.

Results with the methoxy-linked oxazole 1,2,4-oxadiazolidine-3,5-diones are shown in *table I.* All the compounds, with the exception of the methyl-substituted analogs **21** and **22**, were active in the db/db diabetic mouse. The most potent compounds were substituted at the 2-phenyl moiety of the oxazole tail with electron withdrawing groups. The para-substituted (trifluoromethyl)- and (trifluoromethoxy)-

phenyl analogs **27** and **32** were the best compounds of the series. Both compounds reduced plasma glucose levels by 37 and 46%, respectively, at a 20 mg kg^{-1} oral dose. The analogous meta-substituted analogs **28** and **33** were weaker *in vivo*. Substitutions at the 2-phenyl ring may play a critical role in the metabolic stabilization of the phenyl group. The attachment location of the 1,2,4-oxadiazolidine-3,5-dione headpiece to the central aromatic ring of the molecule was also important to the activity. The meta-substituted phenyl (central aromatic group) analog **27** was about five times more potent *in vivo* (db/db) than the para-substituted compound **26**. The most active compounds of the methoxy-linked analogs were also evaluated in the ob/ob diabetic mouse model. The trifluoromethoxy analog **32** was the most potent compound in the ob/ob mouse model. It normalized plasma glucose levels at an oral dose of 20 mg kg^{-1}, and also reduced the plasma glucose levels significantly by 27% at the 5 mg kg^{-1} oral dose. Substitutions on the central aromatic ring with either electron withdrawing or donating groups (i.e. halogen, methoxy) resulted in small reductions of the *in vivo* activity (**23**, **24** vs. **19**; **29**, **30** vs. **27**).

The ethoxy-linked 1,2,4-oxadiazolidine-3,5-diones were also active *in vivo*. Similarly to the methoxy-linked analogs, substitutions on the 2-phenyl moiety of the oxazole tail produced potent compounds *in vivo*. Electronegative groups (trifluoromethyl, halogen) were the best substituents. Compounds **45** and **46** lowered plasma glucose in the db/db mouse by 75, and 71%, respectively, as compared with **36** [15], which lowered plasma glucose by 43% at the oral dose of 100 mg kg^{-1}. The 4-methoxyphenyl analog **40** was active *in vivo*, while the 4-methylphenyl analog **41** was inactive. Metabolic oxidation of the methyl group of **41** might have been a factor to the loss of the *in vivo* activity. Replacement of the 2-phenyl moiety of the oxazole tail with aromatic, hetero-aromatic, and cycloalkyl groups resulted in various decreases in

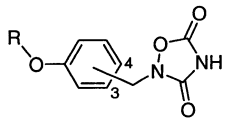
Table III. Chemical and biological data of oxadiazole 1,2,4-oxadiazolidine-3,5-diones [31].[a]

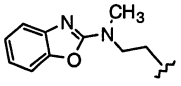
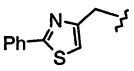
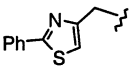
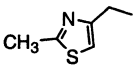
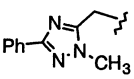


Compound	R^1	*n*	POA [c]	m.p. (°C)	% Decrease in plasma glucose [b]		% Decrease in plasma insulin [b]
					db/db mouse 100 mg kg^{-1}	ob/ob mouse 100 mg kg^{-1}	ob/ob mouse 100 mg kg^{-1}
50	Ph	2	4	152–153	41*		
51	4-CF_3–Ph	2	4	128–129	*i* [d] (20)		
52	Ph	1	4	150–152	30*	47**	87**
53	Ph	1	3	133–135	18*	36* (75 mg kg^{-1})	53** (75 mg kg^{-1})
54	4-CF_3–Ph	1	4	160–162	40*	20*	73**
55	4-CF_3–Ph	1	3	90–92	19*	46** (75 mg kg^{-1})	45* (75 mg kg^{-1})
56	4-F–Ph	1	4	156–157		31*	88**
57	4-F-Ph	1	4	147–149	i		
58	4-Cl–Ph	1	4	168–169	25		
59	4-Cl–Ph	1	3	159–160	*i*		
60	4-OCF_3–Ph	1	4	144–145	38*		
61	4-OCF_3-Ph	1	3	120–121	48*		
62	3,5 di-CH_3–isoxazole	1	3	93–95	*i*		

[a] All compounds were prepared according to *figure 2.*
[b] All values for the drug-treated groups, other than *i*, are significant vs. vehicle-treated mice; * $P<0.05$; ** $P<0.01$.
[c] POA, Point of attachment to the phenyl ring.
[d] *i*, Inactive, generally less than −15% change, $P>0.05$.

Table IV. Chemical and biological data of 1,2,4-oxadiazolidine-3,5-diones.[a]



Compound	R	POA [c]	m.p. (°C)	% Decrease plasma glucose[b]
				db/db Mouse 100 mg kg^{-1}
63		4	155–157	34*
64		4	67–168	28*
65		3	139–140	27*
66		4	165–166	*i* [d]
67		4	175–177	*i*

[a] All compounds were prepared according to *figure 2.*
[b] All values for the drug-treated groups, other than *i*, are significant vs. vehicle-treated mice; *, $P<0.05$; **, $P<0.01$.
[c] POA, Point of attachment to the phenyl ring.
[d] *i*, Inactive, generally less than −15% change, $P>0.05$.

plasma glucose levels. The naphthyl and thienyl analogs **39** and **43** were either weakly active or inactive *in vivo*, while the cyclohexyl and furyl analogs **42** and **44** maintained their *in vivo* activity and were similar to **36**.

Replacement of the oxazole tail with an oxadiazole tail (*table III*) produced several compounds that normalized plasma glucose levels in both, the db/db and ob/ob diabetic mouse models at a 100 mg kg^{-1} oral dose. The phenyl analog **50** was similar to the analogous oxazole analog **36**. Substitutions on the phenyl group of the oxadiazole-tailed compounds were in variance when compared with the oxazole-tailed compounds. Electron withdrawing or donating groups produced compounds that were either similar or weaker to the parent compound (*table III*). The trifluoromethoxy analog **61** normalized plasma glucose levels in the db/db mouse at the oral dose of 100 mg kg^{-1}. Compounds **52**, **53**, **55** and **56** normalized plasma glucose levels in the ob/ob mouse at the oral doses of 75–100 mg kg^{-1}.

Replacements of the oxazole tail with various groups are shown in *table IV*. The benzoxazole analog [17] **63** and the 2-phenyl thiazoles **64** and **65** were active *in vivo* in the db/db mouse, while the 2-methyl-thiazole **66** and 3-phenyl triazole **67** were inactive.

Replacement of the central aromatic region with a benzofuran nucleus produced good *in vivo* compounds (*table V*). The methyl-substituted phenyl analogs **69** and **70** normalized the plasma glucose levels in the db/db mouse at the oral dose of 20 mg kg^{-1}. The naphthyl analog **71** was inactive.

Further replacement of the central aromatic region (*table VI*) with either a naphthalene moiety **73**–**75** or a tetrahydronaphthalene **76** nucleus produced either weakly active (**75**) or inactive *in vivo* (db/db mouse model) compounds. The indane analog **77** decreased plasma glucose levels by 26% at the 100 mg kg^{-1} oral dose.

There have been several reports that the thiazolidinediones are high-affinity peroxisome proliferator activated receptor-γ (PPARγ) agonists, and there is a direct correlation between the *in vitro* potency at the PPARγ receptor and the *in vivo* antihyperglycemic potency in ob/ob mice [30]. Since our compounds possess some structural similarities with the thiazolidinediones-type antihyperglycemic agents, a PPARγ agonistic effect cannot be ruled out as a possible mechanism for the antihyperglycemic properties of these compounds. While our present studies are outside the scope of addressing such a possible mechanism of our compounds, future work will be necessary to elucidate this hypothesis.

4. Conclusions

In summary, we have prepared a series of 1,2,4-oxadiazolidine-3,5-diones and evaluated in two different diabetic animal models of insulin resistance as antihyperglycemic agents. The majority of the methoxy- and ethoxy-linked oxazole 1,2,4-oxadiazolidine-3,5-diones normalized plasma glucose levels at the 100 mg kg^{-1} oral dose in the db/db diabetic mouse model, and several amongst them reduced the

glucose levels at the 20 mg kg^{-1} oral dose. The most potent compounds in the db/db mouse model were also active in the ob/ob diabetic mouse model at the oral dose of 20 mg kg^{-1}. The trifluoromethoxy analog **32** was the most active compound, reducing significantly the plasma glucose levels in the ob/ob mouse model at the 5 mg kg^{-1} oral dose. Oxadiazole-tailed 1,2,4-oxadiazolidine-3,5-diones were also active in both the db/db and ob/ob diabetic mouse models normalizing plasma glucose levels at the 100 mg kg^{-1} oral dose.

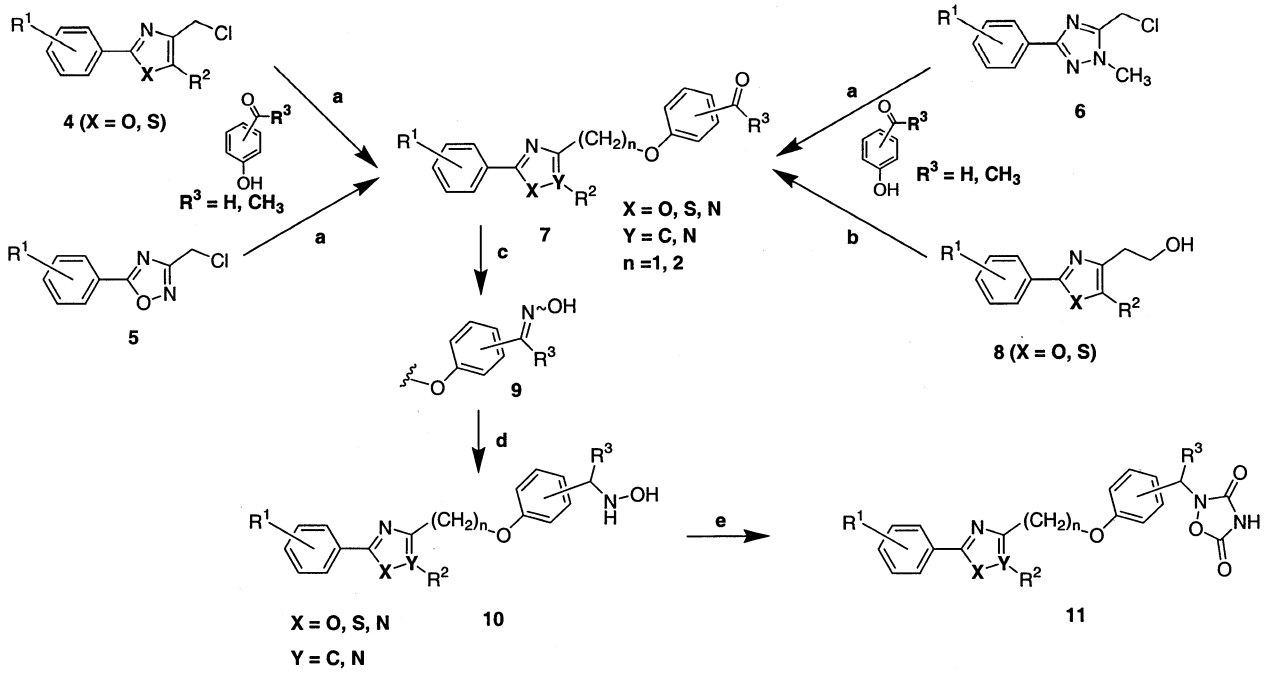
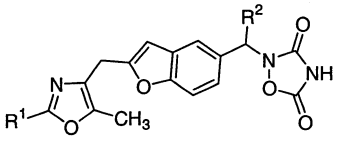


Figure 2. Reagents: (a) K_2CO_3, DMF; (b) Diethyl azodicarboxylate, Ph_3P, THF; (c) $HONH_2{\cdot}HCl$, NaOAc, EtOH, H_2O; (d) $NaCNBH_3$, HCl, MeOH, THF; (e) ClCONCO, THF.

Table V. Chemical and biological data of benzofuran 1,2,4-oxadiazolidine-3,5-diones.[a]



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Compound	R^1	R^2	m.p. (°C)	% Decrease in plasma glucose [b]			% Decrease in plasma insulin [b]
				db/db mouse 100 mg kg^{-1}	db/db mouse 20 mg kg^{-1}	ob/ob mouse 20 mg kg^{-1}	ob/ob mouse 20 mg kg^{-1}
68	Ph	H	189–190	62*	26*	52**	83**
69	4-CH_3–Ph	H	194–196		42*		
70	3-CH_3–Ph	H	180–181		45*		
71	Naphthyl	H	228–229		*i* [c]		
72	Ph	CH_3	174–175	46*			

[a] All compounds were prepared according to *figure 3.*
[b] All values for the drug-treated groups, other than *i*, are significant vs. vehicle-treated mice; $*P<0.05$; $**P<0.01$.
[c] *i*, Inactive, generally less than −15% change, $P>0.05$.

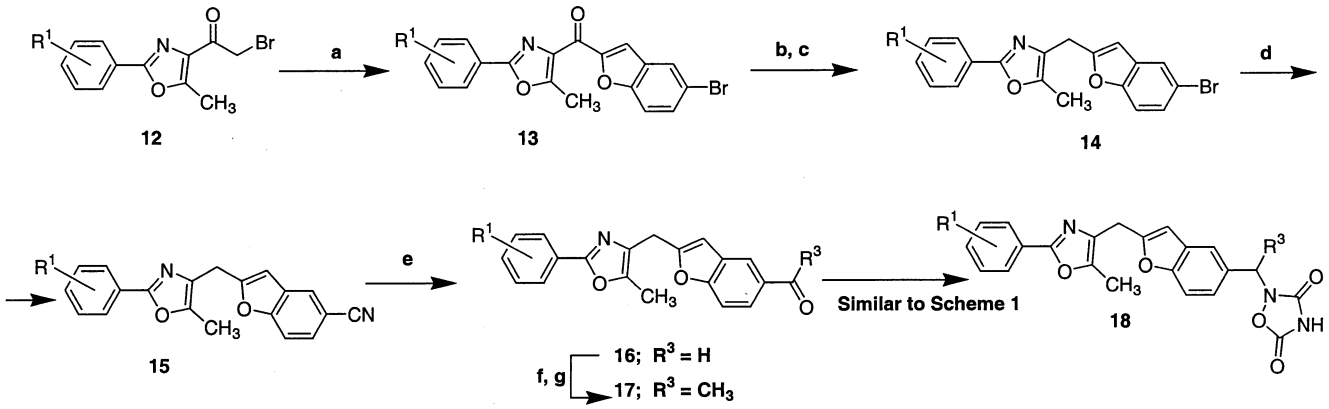


Figure 3. Reagents: (a) $NaOCH_3$, EtOH, 3-Br-2-OH–C_6H_3CHO; (b) $NaBH_4$ MeOH, THF; (c) Et_3SiH, TFA, CH_2Cl_2; (d) CuCN, DMF; (e) Al–Ni, 70% HCO_2H; (f) CH_3MgCl, Et_2O; (g) Jones Reagent, acetone.

5. Experimental protocols

5.1. General chemistry

Melting points were determined in open capillary tubes on a Thomas–Hoover apparatus, and are reported uncorrected ^{1}H-NMR spectra were determined in the cited solvent on a Bruker AM 400 (400 MHz), or a Varian XL-300 (300 MHz) instrument, with tetramethylsilane as an internal standard. Chemical shifts are given in parts per million (ppm) and coupling constants are in hertz. Splitting patterns are designated as follows — s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. The infrared spectra were recorded on a Perkin–Elmer 781 spectrophotometer as KBr pellets or as solutions in chloroform. Mass spectra were recorded on either a Finnigan model 8230 or a Hewlett–Packard model 5995A spectrometer. Elemental analyses (C, H, N) were performed on a Perkin–Elmer 240 analyzer and all compounds are within ±0.4% of theory unless indicated otherwise. All products, unless noted otherwise, were purified by 'flash chromatography' with use of 220–400 mesh silica gel. Thin-layer chromatography was done on silica gel 60 F-254 (0.25-mm thickness) plates. Visualization was accomplished with UV light and/or 10% phosphomolybdic acid in ethanol. Unless otherwise noted, all materials were obtained commercially and used without further purification. All reactions were carried out under an atmosphere of dried nitrogen.

The oxadiazolidinediones described in this paper were synthesized from oxa(thia)zoles **4**, oxadiazoles **5**, triazoles **6**, and alcohols **8** by the following representative procedures. All the required intermediates were prepared as described previously [19–24].

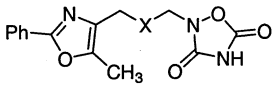
5.1.1. 4-(5-Methyl-2-phenyl-oxazol-4-ylmethoxy)-benzaldehyde (**7**, R^1, $R^3 = H$, $R^2 = CH_3$, $X = O$, $Y = C$, $n = 1$, *para-substituted)*

A mixture of 4-chloromethyl-5-methyl-2-phenyl-oxazole [22, 23] (5.5 g, 26.5 mmol), 4-hydroxybenzaldehyde (3.23 g, 26.5 mmol), potassium carbonate (3.66 g, 26.5 mL) and *N*,*N*-dimethylformamide (80 mL) was stirred at 80°C for 8 h. The mixture was poured into H_2O and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation of the volatiles and purification by flash chromatography on silica gel (eluting solvent hexane/EtOAc 4/1) gave a yellow solid (6.8 g, 86% yield) — m.p. 103–105°C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.46 (s, 3H, CH_3), 5.1 (s, 2H, CH_2), 7.25 (m, 2H, Ar–*H*), 7.51 (m, 3H, Ar–*H*), 7.86–7.93 (m, 4H, Ar–*H*), 9.9 (s, 1H, CHO); IR (KBr, cm^{-1}) 1690 (CO); MS *m*/*e* 294 $(M+H)^+$. Anal. ($C_{18}H_{15}NO_3$) C, H, N.

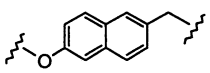
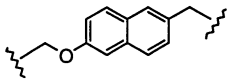
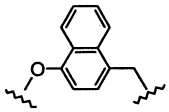
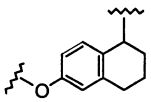
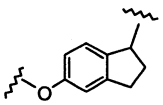
5.1.2. 4-(5-Methyl-2-phenyl-oxazol-4-ylmethoxy)-benzaldehyde oxime (**9**, R^1, $R^3 = H$, $R^2 = CH_3$, $X = O$, $Y = C$, $n = 1$, *para-substituted)*

A solution of sodium acetate (7.27 g, 82.7 mmol) in H_2O (40 mL) was added into a solution of 4-(5-methyl-2-phenyl-oxazol-4-ylmethoxy)-benzaldehyde (6.5 g, 22.2 mmol), hydroxylamine hydrochloride (4.62 g, 66.55 mmol) and ethanol (300 mL). The mixture was stirred at room temperature for 12 h, and then poured into H_2O, acidified with HCl (1N) and extracted with EtOAc. The

Table VI. Chemical and biological data of 1,2,4-oxadiazolidine-3,5-diones.[a]



.

Compound	X	m.p. (°C)	Decrease plasma glucose (%) [b] db/db 100 mg kg^{-1}
73		210–211	*i* [c]
74		207–208	*i*
75		207–209	17*
76		158–159	i
77		162–163	26*

[a] All compounds were prepared according to *figure 2.*
[b] All values for the drug-treated groups, other than *i*, are significant vs. vehicle-treated mice; *, $P<0.05$.
[c] *i*, Inactive, generally less than −15% change, $P>0.05$.

organic extracts were dried over $MgSO_4$. Evaporation of the volatiles and crystallization from acetone/ethyl ether/hexane, gave a white solid (6.1 g, 89% yield) — m.p. 192–193°C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.45 (s, 3H, CH_3), 5.03 (s, 2H, CH_2), 7.06 (m, 2H, Ar–H), 7.49–7.55 (m, 5H, Ar–H), 7.92–7.96 (m, 2H, Ar–H), 8.07 (s, 1H, CH), 10.08 (s, 1H, OH); IR (KBr, cm^{-1}) 3200 (OH); MS m/e 309 $(M+H)^+$. Anal. ($C_{18}H_{16}N_2O_3$) C, H, N.

*5.1.3. N-[4-(5-Methyl-2-phenyl-oxazol-4-ylmethoxy)-benzyl]-hydroxylamine (**10**, R^1, $R^3=H$, $R^2=CH_3$, $X=O$, $Y=C$, $n=1$, para-substituted)*

A solution of HCl (4 N, in dioxane) was added dropwise into a solution of 4-(5-methoxy-2-phenyl-oxazol-4-ylmethoxy)-benzaldehyde oxime (6.0 g, 19.42 mmol) in MeOH (300 mL), THF (60 mL) and methyl orange (indicator, 20 mg) in a rate the reaction pH was maintained at a range 3–4. When a persistent red color was observed, the reaction mixture was poured into H_2O, basified with NaOH (1 N) to pH 9 and extracted with EtOAc. The organic extracts were dried over $MgSO_4$. Evaporation of the volatiles and purification by flash chromatography, on silica gel (eluting solvent EtOAc/MeOH 10/1) gave a yellow solid (5.2 g, 86% yield) — m.p. 109–110°C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.43 (s, 3H, CH_3), 3.8 (s, 2H, CH_2), 4.97 (s, 2H, CH_2), 5. 88 (brs, 1H, NH), 6.98 (d, $J=8.7$ Hz, 2H, Ar–H), 7.2 (s, 1H, OH), 7.24 (d, $J=8.7$ Hz, Ar–H), 7.54 (m, 3H, Ar–H), 7.95 (m, 2H, Ar–H); IR (KBr, cm^{-1}) 3400 (NH), 3250 (OH); MS m/e 311 $(M+H)^+$. Anal. ($C_{18}H_{18}N_2O_3$) C, H, N.

*5.1.4. 2-{4-[(5-Methyl-2-phenyl-1,3-oxazol-4-yl)-methoxy]benzyl}-1,2,4-oxadiazolidine-3,5-dione (**19**)*

N-(Chlorocarbonyl)isocyanate (0.41 mL, 5.16 mmol) was added dropwise into a cold (−5°C) solution of *N*-[4-(5-methyl-2-phenyl-oxazol-4-ylmethoxy)-benzyl]-hydroxylamine (1.6 g, 5.16 mmol), and THF (20 mL). The reaction mixture was stirred for 30 min, poured into HCl (1 N), and extracted with ethyl acetate. The organic extracts were dried over $MgSO_4$. Evaporation of the volatiles and purification by flash chromatography on acid washed (5% H_3PO_4/MeOH) silica gel (hexane/ethyl acetate 2/1) gave a white solid (1.4 g, 72% yield) — m.p. 170–172°C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.44 (s, 3H, CH_3), 4.7 (s, 2H, CH_2), 5.0 (s, 2H, CH_2), 7.04 (d, $J=8.7$ Hz, 2H, Ar–H), 7.27 (d, $J=8.7$ Hz, Ar–H), 7.49–7.53 (m, 3H, Ar–H), 7.93–7.96 (m, 2H, Ar–H), 12.36 (s, brs, 1H, NH); IR (KBr, cm^{-1}) 3450 (NH), 1730 (CO); MS m/e 380 $(M+H)^+$. Anal. ($C_{20}H_{17}N_3O_5$) C, H, N.

*5.1.5. (5-bromo-1-Benzofuran-2-yl)[5-methyl-2-(4-methylphenyl)-1,3-oxazol-4-yl]methanone (**13**, $R^1=CH_3$)*

Sodium methoxide (25% in MeOH, 42.6 g, 197.3 mmol) was added into a mixture of 4-bromo-2-hydroxy-benzaldehyde (39.6 g, 197.3 mmol) and ethyl alcohol (500 mL). The reaction mixture was stirred for 20 min

and then 2-bromo-1-[5-methyl-2-(4-methylphenyl)-1,3-oxazol-4-yl]-1-ethanone (58.0 g, 197.3 mmol) was added. The new mixture was refluxed for 12 h, cooled to 0°C, and the precipitated solid filtered and washed with ethyl alcohol to yield a yellow solid (60.6 g, 78% yield) — m.p. 210–211°C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 2.4 (s, 3H, CH_3), 2.8 (s, 3H, CH_3), 7.4 (d, $J = 8.6$ Hz, 2H, Ar–H), 7.8 (m, 2H, Ar–H), 8.01 (t, $J =$ 8.6 Hz, 2H, Ar–H), 8.2 (s, 1H, Ar–H), 8.64 (s, 1H, CH); IR (KBr, cm^{-1}) 1635 (CO); MS m/e 395 (M^+). Anal. ($C_{20}H_{14}BrNO_3$) C, H, N.

5.1.6. 4-[(5-bromo-1-Benzofuran-2-yl)methyl]-5-methyl-2-(4-methylphenyl)-1,3-oxazole (**14**, $R^1 = CH_3$)

Sodium borohydride (10.4 g, 277 mmol) was added portionwise into a cold (0°C) mixture of (5-bromo-1-benzofuran-2-yl)[5-methyl-2-(4-methylphenyl)-1,3-oxazol-4-yl]methanone (55.0 g, 138.9 mmol), THF (400 mL) and MeOH (500 mL). The mixture was stirred for 2 h, and then poured into water and the precipitated solid filtered and dried to afford a brownish solid (53.0 g). The solid was then taken in dichloromethane (500 mL), and triethylsilane (42.5 mL, 266.3 mmol) and cooled to 0°C. Trifluoroacetic acid (60 mL) was added to the reaction mixture. The mixture was stirred at 0°C for 1 h, and then was allowed to warm up to room temperature where it was stirred for 3 h. The volatiles were removed in vacuo and the residue was taken in ethyl acetate (1000 mL) and washed with water, NaOH (1 N), and brine. The organic extracts were dried over $MgSO_4$. Evaporation of the volatiles and crystallization from acetone/ethyl ether/haxane gave a yellow solid (45.2 g, 85% yield) — m.p. 143–144°C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.33 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 4.07 (s, 2H, CH_2), 6.6 (s, 1H, CH), 7.29 (d, $J = 8.09$ Hz, 2H, Ar–H), 7.35 (dd, $J = 8.7$, 2.07 Hz, 1H, Ar–H), 7.49 (d, $J = 8.7$ Hz, 1H, Ar–H), 7.75 (d, $J = 2.07$ Hz, 1H, Ar–H), 7.79 (d, $J = 8.09$ Hz, 2H, Ar–H); MS m/e 381 (M^+). Anal. ($C_{20}H_{16}BrNO_2$) C, H, N.

5.1.7. 2-{[5-Methyl-2-(4-methylphenyl)-1,3-oxazol-4-yl]methyl}-1-benzofuran-5-carbaldehyde (**16**, $R^1 = CH_3$)

A mixture of 4-[(5-bromo-1-benzofuran-2-yl)methyl]-5-methyl-2-(4-methylphenyl)-1,3-oxazole (30.0 g, 78.5 mmol), copper cyanide (14 g, 157 mmol) and *N*,*N*-dimethylformamide (500 mL) was stirred at 160°C for 24 h. The mixture was then cooled to room temperature, poured into ammonium hydroxide (30%), and extracted with ethyl acetate. Evaporation and crystallization from acetone/ethyl ether/hexane gave a yellow solid (15 g). The solid was taken in formic acid (70%, 170 mL) and Al–Ni (15 g) was added. The mixture was stirred for 4 h, after which, the precipitated solids were filtered and discarded. The filtrate was diluted with ethyl acetate and washed with water, NaOH (2 N), and brine. The organic extracts were dried over $MgSO_4$. Evaporation of the volatiles and purification by chromatography on silica gel (eluting solvent EtOAc/MeOH 10/1) gave a yellow solid (12.1 g, 46% yield) — m.p. 125–126°C; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.33 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 4.1 (s, 2H, CH_2), 6.8 (s, 1H, CH), 7.29 (d, $J = 8.09$ Hz, 2H, Ar–H), 7.7 (d, $J = 8.3$ Hz, 1H, Ar–H), 7.77–7.81 (m, 3H, Ar–H), 8.15 (s, 1Hz, 1H, Ar–H), 10.02 (s, 1H, CHO); IR (KBr, cm^{-1}) 1695 (CO); MS m/e 332 $(M+H)^+$. Anal. ($C_{21}H_{17}NO_3$) C, H, N.

5.2. Biological methods

5.2.1. Determination of plasma glucose lowering in db/db mice

On the morning of day 1, 35 mice male diabetic db/db (C57BL/KsJ) mice (Jackson Laboratories), 2–7 months of age and bodyweight of 50–70 g, were fasted for 4 h, and weighed, and a baseline blood sample (15–30 μl) was collected from the tail-tip of each mouse without anesthesia, and placed directly into a fluoride-containing tube, mixed and maintained on ice (in each individual experiment, ages of the mice were identical between treatment groups (i.e. groups were always matched in any given experiment) and the range of ages as indicated above was actually much narrower than indicated). Food was then returned to the mice. The plasma was separated and levels of glucose in plasma determined by the Abbott VP analyzer. Because of the variable plasma glucose levels of the db/db mice, the five mice having the most extreme (i.e. highest or lowest) plasma glucose levels were excluded and the remaining 30 mice were assigned randomly into seven groups of equivalent mean plasma glucose level (vehicle control, ciglitazone, and five drug groups). On the afternoon of days 1, 2 and 3, vehicle (0.2 mL of 2% Tween 80/saline w/v), control or test drugs were administered (p.o.) to the ad libitum fed mice. On the morning of day 4, the mice were weighed and food removed, but water was available ad libitum. After 3 h, a blood sample was collected and, then, the mice were administered the fourth dose of drug or vehicle. Blood samples were collected again from the

unanesthetized mice 2 and 4 h after drug administration. The plasma was separated and level of glucose in plasma was determined by the Abbott VP analyzer.

To assess the drug activity (considering the well-known variation in plasma glucose values from animal to animal within a litter), the percent change of the animal's plasma glucose level on day 4 (mean of two consecutive bleeds after compound administration; 2- and 4-h samples) compared with the pretreatment plasma glucose level for each individual mouse (from respective level before drug administration (day 1 baseline sample) as follows — (mean of 2- and 4-h samples (day 4)/baseline sample (day 1) multiplied by 100 to give the percentage change).

Analysis of variance (ANOVA) followed by Dunnett's multiple comparison (one-sided) used to estimate the degree of statistical significance of the difference between the vehicle control group and the individual drug-treated groups. A drug considered active, at the specific dosage administered, if the difference of the plasma glucose level had a $P<0.05$. All compounds with $P>0.05$ were reported as inactive.

The positive control, ciglitazone produces a 18–34% decrease in plasma glucose levels at 100 mg kg^{-1} per day×4 days, p.o. ($P<0.05$).

5.2.2. Determination of plasma glucose lowering effect in ob/ob mice

Male or female ob/ob mice (C57BL/6J), ages 2–3 months (40–50 g), were used to assess the effect of compounds on both glucose and insulin lowering. In each study, the ob/ob mice were from the same litter, so they were age-matched and had similar bodyweights and levels of glycemia. Since the degree of glycemia does vary somewhat within the litter, the mice from the litter were randomized into different treatment groups (four groups of ten mice) by bodyweight (we used bodyweight instead of glycemia as a parameter since we found that bodyweight in this age of ob/ob mice is well correlated with glycemic state. From earlier studies, we know that randomization by bodyweight yields tight plasma glucose values for each group). The mice were housed five per cage and were maintained on normal rodent chow with water ad libitum. The mice received the compound daily (morning) by gavage (suspended in 0.5 mL of 0.5% methyl cellulose) for 4 days. The dose of compound administered ranged from 5 to 100 mg kg per day. Bodyweight of animals fed was measured at the beginning of each week and doses for the entire week were calculated using this weight and were expressed in terms of the active moiety of the compound. Control mice received vehicle only.

On the morning of day 4, 4 h after drug administration, blood was collected into the tubes containing sodium fluoride after decapitation under anesthesia. The plasma was isolated by centrifugation and the concentration of glucose was measured enzymatically on an Abbott V.P. analyzer and the plasma concentration of insulin was determined by radioimmunoassay [32]. For each mouse, the percentage change in plasma glucose on day 4 was calculated relative to the mean plasma glucose of the vehicle treated mice. ANOVA, followed by Dunnett's comparison test (one tailed) was used to estimate the significant difference between the plasma glucose values from the control group and the individual compound treated groups. A drug considered active, at the specific dosage administered, if the difference of the plasma glucose level had a $P<0.05$. All compounds with $P>0.05$ were reported as inactive.

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