2 H, C6CH$_2$), 3.4 (s, 2.5 H, H$_2$O), 8.20 (s, br, 2 H, B(OH)$_2$), 12.73 (s, br, 1 H, N1H), 13.40 (s, br, 1 H, N3H); MS (FAB[−], DMPU) 228 [(M − H)$^-$, ^{10}B], 210 [(M − H$_2$O − H)$^-$], 185 [(M − H^{10}BO$_2$ − H)$^-$]. Anal. ($C_7H_{11}N_2O_2S_2B$) (95% ^{10}B, 5% ^{11}B) C, H, N, S, B.

Attempted Reaction of *O,S*-Bis(trimethylsilyl)-5-iodo-2-thiouracil with Butyllithium and Tributyl Borate. 5-Iodo-2-thiouracil (2.45 g, 10 mmol) was refluxed with 0.3 mL (2.4 mmol) of chlorotrimethylsilane and 26 mL of hexamethyldisilazane according to ref 23, and the resulting *O,S*-bis(trimethylsilyl)-5-iodo-2-thiouracil (yield 51%) was purified by Kugelrohr distillation (bp 135 °C, 0.01 mm). Its reaction with butyl lithium and tributyl borate according to the preparation of 1 led to 2-(*n*-butylthio)-5-(trimethylsilyl)uracil: yield 63%; white crystals; mp 117 °C; purification by column chromatography in CHCl$_3$/MeOH 9:1; MS (EI) 256 (M$^+$); ^{1}H NMR (CDCl$_3$) 0.28 (s, 9 H, Si(CH$_3$)$_3$), 0.94–3.17 (m, 9 H, *n*-butyl), 7.84 (s, 1 H, C6H), 12.85 (s, 1 H, N3H); ^{13}C NMR (CDCl$_3$) (−4)–0.5 (q, SiC$_3$), 11.5–33 (m, *n*-butyl), 119.7 (d, $^2J_{C,H}$ = 16.2 Hz, C5), 159.3 (d, $^1J_{C,H}$ = 178 Hz, C6), 163.25–163.58 (d, $^3J_{C,H}$ = 14 Hz + t, $^3J_{C,H}$ = 5.1 Hz, C2), 167.55 (d, $^3J_{C,H}$ = 9.2 Hz, C4).

Attempted Reaction of 5-(Dihydroxyboryl)-1,3-dimethyluracil with Thiourea. 5-Bromo-1,3-dimethyluracil[12] was converted to the 5-dihydroxyboryl derivative as described above (**1d**), increasing the time span between the addition of butyllithium and tributyl borate to 45 min [yield 27%; white crystals; mp 167 °C; MS (EI) 183 (M$^+$); ^{1}H NMR (DMSO-d_6) 3.19 (s, 3 H, N1-CH$_3$), 3.37 (s, 3 H, N3CH$_3$), 8.00 (s, 1 H, C6H), 8.13 (s, 2 H, B(OH)$_2$)].

Reaction of this compound with a 5-fold excess of thiourea and sodium ethylate in ethanol led to 2-thiouracil (yield 74%).

Attempted Rearrangement of 2,4-Bis(benzyloxy)-5-(dihydroxyboryl)pyrimidine. 2,4-Bis(benzyloxy)-5-bromopyrimidine[7] (2 g, 5.4 mmol) was heated with 0.2 g of water-free *p*-toluenesulfonic acid to 165 °C for 3 min. The residue was dissolved in 20 mL of H$_2$O, adjusted to pH = 12, and extracted with ether. Following acidification to pH = 2, two compounds could be extracted with ether. These were purified by column chromatography in CHCl$_3$/MeOH 9:1 and shown to be 1,5-dibenzyluracil [yield 16%; white crystals; mp 132 °C; ^{1}H NMR (CDCl$_3$) 3.49 (s, 2 H, C5CH$_2$), 4.78 (s, 2 H, N1CH$_2$), 7.2 (m, 11 H, C6H + ArH), 9.92 (s, br, 1 H, N3-H)] and 3-benzyluracil [yield 8%, not purified to homogeneity; ^{1}H NMR (CDCl$_3$) 4.98 (s, 2 H, N3CH$_2$), 5.55 (d, 1 H, C5-H), 7.35 (s, 5 H, ArH), 7.62 (d, 1 H, C6-H)].

Animal Distribution Studies. The tumor model used was B16 melanoma (obtained through Dr. J. A. Coderre, Brookhaven National Laboratory) in C57/bl mice. Animals were obtained from Jackson Laboratories, Bar Harbor, ME, or Zentrallaboratorium für Versuchstierzucht, Hannover, FRG. The B16 tumor was propagated in cell culture and implanted into mice by injecting subcutaneously 10^6 cells. The weight of the mice was around 20 g. The tumor size at the time of the experiment was between 0.1 and 0.5 g.

Solutions of 1 and 2 (0.6 and 0.5 mg/mL, respectively) were prepared in 0.1 M Tris base and adjusted to pH = 8. Solutions of 3 and 4 (0.4 and 0.2 mg/mL) were prepared by dissolving the appropriate amount in 0.1 M NaOH and diluting with 10 volumes of water. The pH of this solution was around 9. Of these solutions, 0.5 mL were injected intraperitoneally.

Boron distribution was evaluated with quantitative neutron-capture radiography,[13] with the Brookhaven Medical Research Reactor as neutron source. Frieze-dried sections of 50 μm thickness, obtained in a cryomicrotome, were placed in close contact with a solid-state track detector (Kodak-Pathé LR115, Type 1) and exposed to 10^{12} n cm^{-2}. The exposed film was etched with 10% NaOH for 50–60 min. The tracks that developed were evaluated with an image analyzer and compared to suitable standards.

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Substituted Dihydrobenzopyran and Dihydrobenzofuran Thiazolidine-2,4-diones as Hypoglycemic Agents

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A series of dihydrobenzofuran and dihydrobenzopyran thiazolidine-2,4-diones (compounds **3–26**) was synthesized from the corresponding aryl aldehydes **1** in two steps. These compounds represent conformationally restricted analogues of the novel hypoglycemic ciglitazone. The series was evaluated by hypoglycemic effects in vitro by measuring stimulation of 2-deoxyglucose uptake in L6 myocytes and stimulation of expression of the glucose transporter protein in 3T3-L1 adipocytes. In vivo hypoglycemic effects were evaluated in the genetically obese ob/ob mouse, and structure–activity relationships are discussed. On the basis of this in vivo potency, we have selected the 2(*R*)-benzylbenzopyran derivative to be further studied in a clinical setting.

Diabetes mellitus is a complex, chronic, progressive disease which eventually can adversely affect function of the kidneys, eyes, and nervous and vascular systems. Of the estimated 5.8 million individuals diagnosed with diabetes mellitus in the United States,[1,2] approximately 90% are characterized[3] as non-insulin-dependent (NIDDM, Type II). Most of these NIDDM patients exhibit hyperglycemia, peripheral insulin resistance, and obesity. Besides diet and exercise, current drug therapy for the treatment of diabetes mellitus is aimed at improving glycemic control.[4] The most commonly employed oral hypoglycemics are the sulfonylureas (SU),[5,6] whose mecha-

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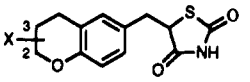
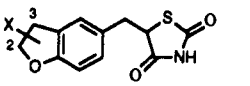
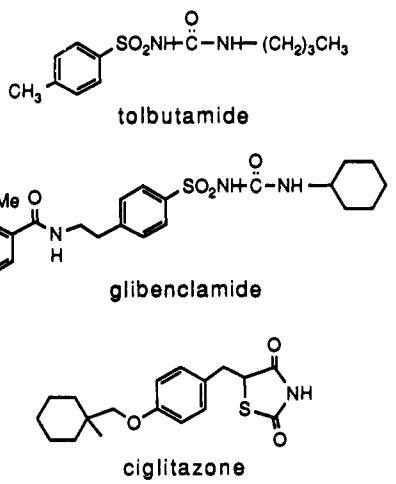
Table I. Physical Data and Yields for Functionalized Benzopyrans and Benzofurans

entry	X	mp, °C	formula	anal.[b]	% yield (3–26)	% yield 2
						
3	2-Ph	131–134	$C_{19}H_{17}NO_3S$	C, H, N	56	66
4	2-CH_2Ph	295–300	$C_{20}H_{18}NO_3SNa$	C, H, N	57	83
5	3,3-$(CH_2)_5$	122–124	$C_{18}H_{21}NO_3S$	C, H, N	50	85
6	2-CH_2-c-hex.	300–305	$C_{20}H_{24}NO_3SNa \cdot {}^1/_4H_2O$	C, H, N	88	44
7	2-c-hex.	155–157	$C_{19}H_{22}NO_3S$	C, H, N	38	78
8	2,2-$(CH_2Ph)_2$	220–240	$C_{27}H_{24}O_3NSNa \cdot H_2O$	C, H, N	82	73
9	2-$CH_2C_6H_4$-4-OBn	270–272	$C_{27}H_{24}O_4NSNa$	C, H, N	77	65
10	2-$CH_2C_6H_4$-4-OH	198–200	$C_{20}H_{19}NO_4S$	C, H, N	(35)[a]	
11	2-$CH_2C_6H_4$-4-OMe	245–255	$C_{21}H_{20}NO_4SNa \cdot {}^1/_2H_2O$	C, H, N	83	76
12	2,2-$(CH_2)_5$	>280	$C_{18}H_{20}NO_3SNa$	C, H, N	86	75
13	2,2-Et_2	248–255	$C_{17}H_{20}NO_3SNa$	C, H, N	90	46
14	2,2-$(CH_2)_6$	119–123	$C_{19}H_{23}NO_3S$	C, H, N	54	50
15	2,2-$(CH_2)_4$	>220	$C_{17}H_{18}NO_3SNa$	C, H, N	83	53
16	2-CH_2(2-Pyr)	255–265	$C_{19}H_{17}N_2O_3SNa \cdot {}^1/_2H_2O$	C, H, N	30	98
17	2(*R*)-CH_2Ph	288	$C_{20}H_{18}NO_3SNa$	C, H, N	68	100
18	2(*S*)-CH_2Ph	286	$C_{20}H_{18}NO_3SNa$	C, H, N	65	100
						
19	2-Ph	143–147	$C_{18}H_{15}NO_3S$	C, H, N	42	62
20	2-CH_2Ph	130–136	$C_{19}H_{17}NO_3S$	C, H, N	30	79
21	3-Ph	68–74	$C_{18}H_{15}NO_3S$	C, H, N	31	50
22	2,2-$(CH_2)_5$	157–160	$C_{17}H_{19}NO_3S$	C, H, N	55	69
23	3-CH_2Ph	145 dec	$C_{19}H_{16}NO_3SNa \cdot H_2O$	C, H, N	25	30
24	2-CH_2-c-hex.	280–284	$C_{19}H_{22}NO_3SNa \cdot {}^1/_4H_2O$	C, H, N	67	99
25	2-c-hex.	>300	$C_{18}H_{20}NO_3SNa$	C, H, N	65	98
26	2-(1-Me-c-hex.)	291–293	$C_{19}H_{22}NO_3SNa$	C, H, N	89	83

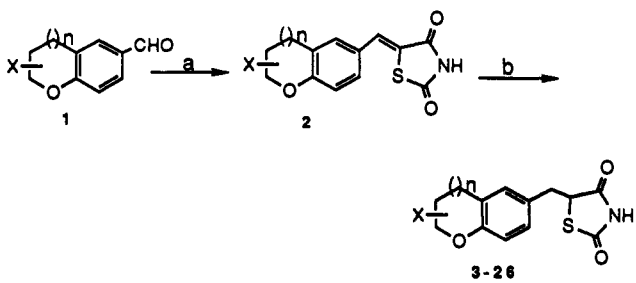
[a] Prepared from 11. [b] Compounds gave satisfactory analyses (±0.4%).

nism of action probably involves insulin release.[7] There is evidence, however, that the sulfonylureas additionally have extrapancreatic actions which help alleviate the insulin resistance associated with NIDDM.[8,9] Both of the first generation SU, e.g., tolbutamide,[10] and the second generation SU, e.g., glibenclamide,[11] are valuable therapies but possess disadvantages such as primary or secondary failure of efficacy as well as the potential for induction of hypoglycemia (Chart I). Numerous other non-sulfonylurea hypoglycemic compounds have been clinically investigated in NIDDM, but none of these has been sufficiently efficacious or well-tolerated in man to have reached the market.[12,13] Many of these newer agents reduce elevated glucose levels in animal models by non-insulin-releasing mechanisms and have been the subject of several recent reviews.[14,15]

Chart I



Scheme I[a]



[a] (a) 2,4-Thiazolidinedione/NaOAc/Δ; (b) H_2/Pd–C.

In 1982, Takeda disclosed a novel series of (4-substituted-benzyl)thiazolidine-2,4-diones that reduce insulin

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Table II. In Vitro and in Vivo Activity of Functionalized Benzopyrans and Benzofurans

	2-DG uptake stim,[a] L6 myocytes			GT fold stim,[b] 3T3-L1 adipocytes		% glucose normalization[c] ob/ob mouse			
	3 μM	10 μM	30 μM	10 μM	30 μM	25 mg/kg	10 mg/kg	5 mg/kg	2.5 mg/kg
3	0.78*	0.95	1.15*	1.05**	1.37	70*	0		
4	0.91*	1.29*	1.14*	1.14	1.48	91*	70[d,*]	48[e]	29
5	1.15	1.27*	1.38*	0.80	1.23	68*	6		
6	1.14	1.55*	0.68*	1.02**	1.28	7			
7	1.28*	2.08*	2.23*	1.03**	1.23	69*	75	65*	5
8	0.73*	0.86*	0.44*	1.03**	1.23	12			
9	1.42*	1.53*	1.34*	1.48	1.59	95*	100*	73*	19
10	1.04	1.24*	1.03	1.25	1.34	100*	55		
11	0.92	1.15	0.69*	1.08**	1.21	85*	68	10	
12	0.97	1.52*	1.22*	0.97**	1.20	35			
13	1.01	1.33*	1.38*	1.08**	1.21	24			
14	1.32*	0.99	0.43*	0.94**	1.12**	0			
15	0.79**	1.06	1.18*	0.95**	1.10**	52	64	50	0
16	0.76*	0.96	0.96	1.48	1.42	74*	49		
17	0.77*	0.91*	1.27*	1.13	1.24	88*	90[f,*]	73[g,*]	31
18	0.77*	0.90*	1.23*	1.13	1.17	73*	47[h]	41[i]	
19	0.76*	0.99	1.30*	1.14**	1.42	85*	6		
20	0.91*	1.11	1.51*	1.34	1.51	100*	95*	65*	0
21	1.05	1.24*	1.42*	1.13	1.19	32			
22	0.85*	1.28*	1.62*	1.15	1.36	56			
23	0.85*	1.25*	1.40*	1.09*	1.31	0			
24	0.88*	1.39*	1.71*	1.14	1.41	19			
25	1.10*	1.85*	1.66*	1.25	1.42	23			
26	0.80*	0.93	1.67*	1.11**	1.22	80*	33		
ciglitazone	0.82*	1.02	0.68*	1.49	1.51	84*	52*		

[a] Insulin (1 μM) stimulation is ca. 50% (standardized to 1.00). Active compounds are ≥1.00 and significant (* = $p < 0.05$ when compared to insulin). [b] Fold stimulation of glucose transporter protein. All values are the average of two runs done in triplicate and are significant ($p < 0.05$) unless indicated with **. [c] Normalization (i.e. 100%) = Ciglitazone effect at 50 mg/kg (* = $p < 0.05$ when compared to vehicle control). [d] Average of five separate runs ±17%. [e] Average of three separate runs ±32%. [f] Average of four separate runs ±16%. [g] Average of three separate runs ±11%. [h] Average of five separate runs ±16%. [i] Average of four separate runs ±21%.

resistance or potentiate insulin action in genetically diabetic and/or obese animals.[16] The prototypical agent, ciglitazone, was shown to lower plasma glucose levels in animal models of Type II diabetes but not in nondiabetic animal models.[17] Since that time, numerous reports of additional (substituted-benzyl)thiazolidinediones have appeared marking an effort to identify more potent and better tolerated derivatives.[18-24] We report herein our efforts toward the synthesis of agents of this class which are conformationally restricted in the lipophilic tail region by the introduction of dihydrobenzofuran and dihydrobenzopyran rings.

Chemistry

The thiazolidinediones in Table I were readily prepared from the corresponding aryl aldehydes **1** by the two-step procedure shown in Scheme I. Fusion of the neat aryl aldehydes **1** with 2,4-thiazolidinedione (1.5 equiv) and anhydrous sodium acetate (2.5 equiv) at 140–190 °C for 30 min afforded the olefinic thiazolidinediones **2** in excellent yield (44–100%). Olefins **2** were purified by simple sequential trituration with water followed by ethyl acetate or ether and then subjected to catalytic hydrogenation in acetic acid using 10% palladium/carbon. The resultant thiazolidinediones **3–26** were purified by silica gel chromatography or by crystallization to afford analytically pure material in yields ranging from 30 to 90%. Low-melting solids were converted to their sodium salts with either sodium methoxide or sodium 2-ethylhexanoate.

Aryl aldehydes **1** were prepared from the appropriately substituted dihydrobenzofuran or dihydrobenzopyran precursors[19] by standard formylation procedures using phosphorus oxychloride/*N*-methylformanilide,[25] α,α-dichloromethyl ether/titanium tetrachloride,[26] or hexa-

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methylenetetramine/trifluoroacetic acid.[27] Alternatively, the corresponding aryl bromide was converted to the aldehyde by transmetalation with *n*-butyllithium followed by quenching the intermediate aryllithium with dimethylformamide. All of the derivatives in Table I were simple racemates or racemic mixtures of diastereomers with the exception of entries **17** and **18**. Analogue **4** was resolved at C-2 on the dihydrobenzopyran (entries **17** and **18**) in order to examine the effects of stereochemistry at this position. Resolution was effected by fractional crystallization of the α-methylbenzylamine salt of (*R*,*S*)-2-(phenylmethyl)chroman-6-carboxylic acid,[19a] followed by conversion to the aldehyde and then thiazolidinedione.

Biological Procedures

(1) In Vivo Hypoglycemic Activity. Six to eight week old C57 BL/6J-ob/ob mice (obtained from Jackson Laboratories, Bar Harbor, ME) were housed five per cage under standard animal-care practices. After a 1-week acclimation period, the animals were weighed and 25 μL of blood was collected via the retroorbital sinus prior to any treatment. The blood sample was immediately diluted 1:5 with saline containing 2% sodium heparin and held on ice for glucose analysis. Animals were then dosed daily for four days with drug or vehicle. All drugs were administered by oral gavage, once daily, in a vehicle consisting of 0.25% (w/v) methylcellulose in water with no pH adjustment (0.1 mL of solution per 20 g of animal weight). Animals were bled 24 h after the fourth administration of drug or vehicle (via the retroorbital sinus) for blood glucose levels. The weight of each animal was recorded on days 1 and 5 of the treatment. The freshly collected samples (125 μL in 330-μL tubes) were centrifuged for 2 min at 10000*g* at room temperature. A 50-μL sample was analyzed for glucose by the Abbott VP Super System Analyzer,[28] using the A-gent[28] glucose UV reagent system[29] (hexokinase method using 100, 300, and 500 mg/dL standards). Ciglitazone was dosed at 50 mg/kg as a positive control, and results are reported in Table II as percent glucose normalization compared to the standard ciglitazone-treated group (100% at 50 mg/kg) and the vehicle-treated group (0%).

(2) In Vitro Stimulation of 2-Deoxyglucose Uptake in L6 Myocytes. The cell line employed, L6, is rat skeletal myoblasts that spontaneously fuse and differentiate into myocytes upon growing to confluence. Deoxyglucose uptake in the differentiated cells was stimulated approximately 50% by the maximally effective insulin concentration of 1 μM, which served as the positive control. Results in Table II are reported compared to insulin (standardized as 1.0). Cells were grown in six-well cluster plates (35-mm wells) in MEMalpha + 2% FBS for 14 days. On the day of the transport experiment, the cells were depleted of serium for 5 h, which greatly reduced the basal rate of transport. During this period the cells were triturated with drug for the 5 h, or where appropriate, with

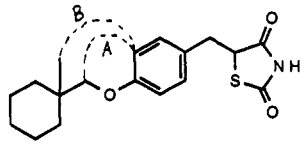

Figure 1.

insulin for the final 40 min of this period. Transport was measured in Dulbecco's phosphate-buffered saline (+Ca, Mg) containing 0.1 mM [1-^{14}C]2-deoxyglucose. Cells were incubated at room temperature for 10 min. Uptake was terminated by aspirating the media and washing the cells three times with ice-cold saline. The cells were dissolved in 0.1 M NaOH and counted in a scintillation counter.

(3) In Vitro Stimulation of Glucose Transporter Protein. Affinity-purified rabbit antibodies against the purified human erythrocyte glucose transporter (GLUT 1) is a preparation that has been described by Schroer.[32] [^{125}I]goat anti-rabbit IgG was from New England Nuclear.

Cell Culture. 3T3-L1 cells were carried as fibroblasts and differentiated into adipocytes as previously described.[33] The adipocytes were used between days 7 and 11 after initiation of differentiation. Test compounds were dissolved in neat dimethyl sulfoxide (DMSO) and added to adipocytes in Dulbecco's modified Eagle medium containing 10% fetal bovine serum such that the final DMSO concentration was 0.1%. This medium was replaced with fresh drug-containing medium after 24 h and incubation continued for an additional 24 h, at which time the cells were extracted for glucose transporter quantitation. Control cells received 0.1% DMSO alone, which was without effect on glucose transporter expression.

Quantitative Glucose Transporter Dot Immunoblotting. 3T3-L1 adipocytes on 35-mm dishes were extracted at room temperature for 30 min on an orbital shaker with 0.6 mL of 4% Triton X-100/5 mM EDTA/20 mM dithiothreitol, pH 7.0, that contained protease inhibitors (1 μg/mL pepstatin A, 0.5 μg/mL leupeptin, 10 μM 1-[L-*trans*-N^{α}-(epoxysuccinyl)leucylamido]-(4-guanidino)butane, and 200 μM phenylmethylanesulfonyl fluoride). Cell extracts (6 μL) were applied directly to nitrocellulose (Schleicher and Schull, BA83, 0.2 μm) that had been washed in H_2O and dried. The nitrocellulose was incubated with the affinity-purified glucose transporter antibodies (0.5 μg/mL) and then with ^{125}I-labeled goat antibodies against rabbit IgG at 2×10^5 dpm/mL as described by Gibbs.[34] For quantitation of the immunoblots, the labeled spots were cut out and counted in a γ-counter. Various amounts of extract from basal cells were dotted on each immunoblot to construct a standard curve. The relative amount of glucose transporter in each cell extract was then determined by comparing the ^{125}I cpm to the standard curve, which was linear over the range of transporter concentrations used in the present study.

Results and Discussion

The lead compound, ciglitazone, in our hands normalized blood glucose in ob/ob mice (288 ± 12 mg/dL fell to 157 ± 5 mg/dL) to the level of the lean littermates (ob/−, 177 ± 15 mg/dL) at 50 mg/kg, while causing a 52% reduction (relative to the 50 mg/kg dose) at 10 mg/kg. Although the mechanism of this class of compounds is not

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fully understood, should they bind to a unique receptor, one strategy to increase the potency could involve constraining the freely rotating (methylcyclohexyl)methyl moiety within the boundaries of a fused ring. One can envision two distinct structural forms that would restrict the mobility of this lipophilic side chain (Figure 1). The first, and conceptially simplest, involves the annulation of a ring onto the phenyl group in which the newly appended ring contains the ether oxygen and methylene carbon of ciglitazone. An example of this type of compound (type A) is exemplified by **26**. Another variant (type B) would involve not only the ether oxygen and methylene carbon of ciglitazone but also the quaternary and methyl carbons to be annealed in the fused ring described by **5**. Following these considerations, the compounds detailed in Table II were prepared and their comparative potency in the ob/ob mouse discussed.

Examination of the type B compounds, in which the quaternary carbon is within the fused ring (entries **5**, **8**, **12–15**, and **22**), shows a marked effect of the substituents on the heterocyclic ring on the potency of these compounds. The most potent compound in vivo from within this limited series is **15**, with a fused cyclopentane ring at C2 of the benzopyran nucleus. Expansion of the spiro cyclic ring to either six (**12**) or seven (**14**) carbons caused a marked decrease in activity. If the spiro cyclopentane ring of **15** is broken open to afford a *gem*-diethyl moiety (**13**), activity is dramatically lowered. The same loss in activity is seen by appending an additional benzyl at C-2 to give the *gem*-dibenzyl compound **8**. It is intriguing to note that migration of the spiro cyclohexane from C2 to C3 of the benzopyran ring (**5**) increases the potency, as does ring contraction from a C2 spiro substituted benzopyran to a C2 spiro substituted benzofuran (**22**).

If we now turn our attention to the type A compounds, we see an equally striking effect of the fused heterocyclic ring substituents on activity. The prototypical compound in this series, **26**, is marginally more potent than either of its type B analogues entries **5** and **22**. Removal of the quaternary methyl group then affords benzofuran **25**, a compound with dramatically lower activity. Aromatization of the pendent cyclohexane ring to give **19** then returns the potency to the vicinity of the prototype. Ring expansion of the benzofuran ring of **25** to give the benzopyran affords one of the most potent compounds in this series, **7**. Surprisingly, aromatization of the cyclohexane ring to give the 2-phenylbenzopyran **3**, is coincident with a drop in activity, the potency being comparable to that of the benzofuran analogue **19**. Homologation of the side chain by one carbon to afford the cyclohexylmethyl analogues in both the benzofuran and benzopyran series, entries **24** and **6**, respectively, was concomitant with a less potent compound. This time aromatization of the side chain cyclohexyl moiety in the side chain to give the 2-benzylbenzofuran (**20**) and 2-benzylbenzopyran (**4**) compounds was accomplished with a dramatic increase in activity. Potency was greatly diminished by migration of the lipophilic side chain from the 2 to the 3-position of the benzofuran ring (entries **21** and **23**).

As **4** now respresents an approximately 2-fold increase in potency over ciglitazone, we examined permutations of this compound in the hope of finding a further increase in activity. Preparation of the optical antipodes at the benzopyran center of **4** affords **17** and **18**. In a side-by-side comparison, entry **17**, the 2(*R*) compound, displayed a slight potency advantage over the 2(*S*) compound **18**. None of the analogues **3–26** were resolved at the thiazolidinedione stereocenter since it was precisely shown that this center in ciglitazone rapidly epimerizes.[17d] Further, ^{1}H NMR experiments showed that in aqueous alkali (D_2O, pH = 9), the stereogenic center (C-5) of the thiazolidine-2,4-dione of **4** rapidly equilibrated ($t_{1/2}$ = 3 h at 37 °C for exchange with deuterium from solvent (D_2O)). In vitro and in vivo experiments with the C-5 (thiazolidine-2,4-dione) deuterated **4** demonstrated that loss of deuterium occurred within minutes under physiological conditions, thus resolution of this center was not warranted.

If the benzyl moiety of racemic **4** is replaced with a 2-pyridylmethyl group (entry **16**), potency is clearly decreased. Substitution on the pendant phenyl group at the 4-position with either a hydroxy (**10**) or a methoxy (**11**) group lowers activity; however, with the 4-benzyloxy derivatives (**9**) a minor increase in potency was seen.

We then examined the above compounds for their ability to stimulate expression of the glucose transporter protein in vitro as detailed in Table II. The most active compounds in this assay (**4**, **9**, **10**, **16**, **20**, **22**, **24**, and **25**) span the range from the most to least potent compounds in the in vivo evaluation. Interestingly, all of the compounds except for **22** are of type A structural class. Noteworthy also, is the good (60%) correlation between the eight most active GT-stimulating-compounds and the eight most active in vivo compounds. Lack of complete concordance between the in vivo and in vitro results may arise from differences in pharmacokinetic and metabolic parameters.

The ability of these compounds to stimulate the uptake of 2-deoxyglucose in vitro was investigated (Table II). The media that this assay was run in was serum free, and caused a deviation from the expected dose-dependent response for some compounds, presumably due to cytotoxicity at the higher concentrations. This effect typically was not seen in the glucose transporter assay, which used serum, to which >95% of these drugs are bound, leaving less free drug to interact with the cell. For this reason, we have arbitrarily defined the most potent compounds as those that at the 3 μM concentration significantly stimulate deoxyglucose uptake greater than insulin. The four compounds that fit into this category are **7**, **9**, **14**, and **25**. As in the case of glucose transporter protein stimulation, they span the range from among the most to the least potent in vivo. It is interesting to note that the two compounds active in both in vitro assays (**9** and **25**) are at opposite ends of the potency range that we have observed in the ob/ob mouse model.

In summary, conformational restriction of the lipophilic tail region of ciglitazone by the introduction of dihydrobenzofuran and dihydrobenzopyran rings has led to compounds with improved in vitro and in vivo activity. Within the compounds discussed, we could find no correlation between physiochemical parameters and activity. On the basis of in vivo potency as an antihyperglycemic agent in the ob/ob mouse, as well as additional profiling in alternative animal models, the resolved dihydrobenzopyran **17** (englitazone) was selected for clinical studies.[31,35]

Experimental Section

Melting points were taken with a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were carried out by the Analytical Department of Pfizer Central Research, and results obtained for specified elements are within ±/−0.4% of the theoretical values unless otherwise denoted. ^{1}H NMR spectra of deuteriochloroform or DMSO-d_6 solutions (internal standard TMS, δ 0 or the solvent was utilized as an internal standard and

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deuterium lock) were recorded on Varian A-60 or Varian XL-300 spectrometers. Low-resolution and high-resolution mass spectra were obtained on Finnigan 4510 and AEI MS-30 instruments, respectively. Infrared spectra were recorded on a Perkin-Elmer 283 spectrophotometer.

General Procedure for (Arylmethylene)-2,4-Thiazolidinediones. 5-[(2-Benzyl-3,4-dihydro-2*H*-1-benzopyran-6-yl)methylene]thiazolidine-2,4-dione. An intimate mixture of 2-benzyl-6-formyl-3,4-dihydro-2*H*-1-benzopyran (1.5 g, 5.9 mmol), 2,4-thiazolidinedione (876 mg, 7.40 mmol), and anhydrous sodium acetate (1.20 g, 14.8 mmol) were heated in an oil bath at 140 °C with stirring. The reaction mixture melted and then resolidified after 10–15 min and heating was continued for an additional 5–10 min. The resulting mixture was cooled to room temperature and triturated with water (50 mL) followed by acetone (25 mL). The solids were collected by filtration, washed with ether, and dried to afford the title compound (1.75 g, 83%) as a yellow solid; mp 183–184 °C.

General Procedure for (Arylmethyl)-2,4-thiazolidinediones. 5-[(2-Benzyl-3,4-dihydro-2*H*-1-benzopyran-6-yl)-methyl]thiazolidine-2,4-dione. To a solution 5-[(2-Benzyl-3,4-dihydro-2*H*-1-benzopyran-6-yl)methylene]thiazolidine-2,4-dione (1.75 g, 5.00 mmol) and acetic acid (225 mL) was added 1.7 g of 10% palladium-on-carbon (sulfur resistant, Englehard) and the reaction mixture was hydrogenated in a Parr shaker at 50 psi (3.5 bars) overnight at room temperature. The mixture was filtered and the filtrate concentrated in vacuo. The residue was diluted with ethyl acetate (250 mL) and washed with saturated sodium bicarbonate (100 mL), brine (100 mL), and dried ($MgSO_4$). Evaporation of the solvent in vacuo afforded the title compound (1.0 g, 57%), which was isolated as a foam.

Conversion to the sodium salt was accomplished as follows: the thiazolidine-2,4-dione (1.0 g, 2.8 mmol) was dissolved in ethyl acetate (30 mL) and sodium 2-ethylhexanoate (465 mg, 2.8 mmol) was added in one portion. After stirring overnight, the solvent was removed under reduced pressure and the resultant paste was triturated with ether, filtered, and washed with hexanes to afford the sodium salt (840 mg, 84%) as a white solid: mp 295–300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60 (m, 1 H), 1.93 (m, 1 H), 2.52 (dd, J = 11, 14 Hz, 1 H), 2.70 (m, 2 H), 2.86 (dd, J = 6, 14 Hz, 1 H), 3.00 (dd, J = 6, 14 Hz, 1 H), 3.26 (dd, J = 4, 14 Hz, 1 H), 4.06 (dd, J = 4, 11 Hz, 1 H), 4.20 (m, 1 H), 6.58 (d, J = 9 Hz, 1 H), 6.87 (m, 2 H), 7.3 (m, 5 H).

Preparation of Substituted 5-Formyl-2,3-dihydro-1-benzofuran and 6-Formyl-3,4-dihydro-2*H*-1-benzopyran Starting Materials. Preparation of the aldehyde precursors to thiazolidinediones **3–7**, **9**, **11**, and **17–26** are described in ref 19.

2,2-Dibenzyl-6-formyl-3,4-dihydro-2*H*-1-benzopyran. Benzylmagnesium chloride (25 mL of 2.0 M solution in THF, 50 mmol) was added to THF (50 mL) and cooled to −78 °C. 6-Bromo-2-chromanone[19] (2.27 g, 10 mmol) in THF (25 mL) was added to the cooled solution over 5 min and the solution was stirred at −78 °C for 15 min and allowed to warm to room temperature over 2 h. The reaction mixture was quenched with saturated NH_4Cl (50 mL) and extracted with ether (2 × 150 mL). The combined organics were washed with 10% $NaHCO_3$ (50 mL) and brine (50 mL) and dried ($MgSO_4$). The solvent was removed in vacuo to afford the crude bisbenzyl adduct, which was isolated as a solid without purification. The crude product and *p*-toluenesulfonic acid monohydrate (400 mg) were heated to relux in toluene (400 mL) with a Dean–Stark trap for 15 h. The solution was cooled, diluted with ether (400 mL), and washed with 10% $NaHCO_3$ (100 mL) and brine (100 mL), and dried ($MgSO_4$). The solvent was removed in vacuo and the residue triturated with ether/hexane to afford 1.4 g (35%) of 6-bromo-2,2-dibenzyl-3,4-dihydro-2*H*-1-benzopyran; mp 109–110 °C.

6-Bromo-2,2-dibenzyl-3,4-dihydro-2*H*-1-benzopyran (1.4 g, 3.56 mmol) was dissolved in THF (25 mL) and cooled to −78 °C. *n*-Butyllithium (1.55 mL of 2.3 M solution in hexane, 3.56 mmol) was added and the solution was stirred at −78 °C for 45 min. Dimethylformamide (0.33 mL, 4.25 mmol) was added and the solution was then stirred for 2 h at −78 °C. HCl (5%, 25 mL) was added and the mixture warmed to room temperature and extracted with ethyl acetate (2 × 100 mL). The combined organics were washed with brine (100 mL) and dried ($MgSO_4$). The solvent was removed in vacuo and the residue purified on silica gel using hexane/ethyl acetate (3:1) to afford 1.1 g (90%) of 2,2-dibenzyl-6-formyl-3,4-dihydro-2*H*-1-benzopyran as a gum: ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.68 (t, J = 6 Hz, 2 H), 2.75 (t, J = 6 Hz, 2 H), 2.88 (s, 4 H), 6.88 (d, J = 8 Hz, 1 H), 7.10 (s, 10 H), 7.6 (m, 2 H), 9.80 (s, 1 H); MS m/e 342.

5-[(2-(4-Hydroxybenzyl)-3,4-dihydro-2*H*-1-benzopyran-6-yl)methyl]thiazolidine-2,4-dione (10). Benzyl ether **9** (1.0 g, 2.2 mmol) was suspended in a mixture of acetic acid (30 mL) and 48% HBr (15 mL) and heated to reflux for 1.5 h and then cooled to room temperature and poured onto ice. The mixture was extracted with ethyl acetate (2 × 100 mL), and the combined organics were washed with water (100 mL), 10% $NaHCO_3$ (until neutral), and brine (100 mL) and dried ($MgSO_4$). The solvent was removed in vacuo and the residue was crystallized from hexane/ethyl acetate to afford 282 mg (35%) of the title compound **8**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.6 (m, 1 H), 1.9 (m, 1 H), 2.6–2.8 (m, 3 H), 2.8–3.0 (m, 2 H), 3.3 (dd, J = 14, 3 Hz, 1 H), 4.1 (m, 1 H), 4.8 (m, 1 H), 6.6–6.7 (m, 4 H), 6.9 (m, 2 H), 7.05 (d, 2 H), 9.2 (bs, 1 H); MS m/e 369.

Spiro[6-formyl-3,4-dihydro-2*H*-1-benzopyran-1′-cyclopentane]. Cyclopentanespiro-2-(4-chromanone)[30] (50.5 g, 0.25 mol) was hydrogenated in a Parr shaker at 50 psi (3.5 bar) at room temperature for 4 h in MeOH (600 mL) containing gaseous HCl (50 g) using 50 g of 5% palladium-on-carbon (50% water weight). The catalyst was removed by filtration through Celite and the solvent was removed in vacuo. The residue was dissolved in ethyl acetate (1000 mL), and washed with ater (500 mL) and brine (500 mL), and dried (Na_2SO_4). The solvent was removed in vacuo and the crude product was purified on silica gel using hexane/ether (3:1) as eluent to afford 28.6 g (61%) of spiro[3,4-dihydro-2*H*-1-benzopyran-2,1′-cyclopentane] as an oil: MS m/e 188. The following benzopyrans were prepared in a similar manner: **spiro[3,4-dihydro-2*H*-1-benzopyran-2,1′-cyclohexane]**: oil; MS m/e 202. **Spiro[3,4-dihydro-2*H*-1-benzopyran-2,1′-cycloheptane]**: oil; MS m/e 216. **2,2-Diethyl-3,4-dihydro-2*H*-1-benzopyran**: bp 110 °C (0.35 mmHg); MS m/e 204.

Spiro[3,4-dihydro-2*H*-1-benzopyran-2,1′-cyclopentane] (18.8 g, 0.100 mol) was dissolved in *N*-methylformanilide[25] (27 mL, 0.2 mol) at room temperature. Phosphorus oxychloride (18.6 mL, 0.200 mol) was added dropwise over 0.5 h (slight exotherm). The reaction mixture was stirred at room temperature for an additional 0.5 h and then heated at 80 °C for 1 h. After cooling, the reaction mixture was diluted with ethyl acetate (500 mL), with 15% NaOAc (3 × 250 mL), water (250 mL), and brine (250 mL), and dried (Na_2SO_4). The solvent was removed in vacuo and the residue was purified on silica gel using methylene chloride as eluent to afford 17.4 g (81%) of spiro[6-formyl-3,4-dihydro-2*H*-1-benzopyran-2,1′-cyclopentane] as an oil: ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.6–2.1 (m, 10 H), 2.83 (t, J = 7 Hz, 2 H), 6.72 (d, J = 9 Hz, 1 H), 7.5–7.7 (m, 2 H), 9.79 (s, 1 H). The following aldehydes were prepared in a similar manner: **Spiro[6-formyl-3,4-dihydro-2*H*-1-benzopyran-2,1′-cyclohexane]**: oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.3–1.8 (m, 12 H), 2.76 (t, J = 7 Hz, 2 H), 6.84 (d, J = 9 Hz, 1 H), 7.54 (s, 1 H), 7.55 (d, J = 9 Hz, 1 H), 9.76 (s, 1 H); MS m/e 230. **Spiro[6-formyl-3,4-dihydro-2*H*-1-benzopyran-2,1′-cycloheptane]**: oil; ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.2–2.0 (m, 14 H), 2.79 (t, J = 7 Hz, 2 H), 6.80 (d, J = 9 Hz, 1 H), 7.5 (m, 2 H), 9.83 (s, 1 H); MS m/e 244. **2,2-Diethyl-6-formyl-3,4-dihydro-2*H*-1-benzopyran**: oil; ^{1}H NMR (60 MHz, $CDCl_3$) δ 0.95 (t, J = 6 Hz, 6 H), 1.60 (t, J = 7 Hz, 2 H), 1.79 (q, J = 6 Hz, 4 H), 2.78 (t, J = 7 Hz, 2 H), 6.77 (d, J = 9 Hz, 1 H), 7.5 (m, 2 H), 9.80 (s, 1 H); MS m/e 218.

6-Formyl-2-(2-pyridylmethyl)-3,4-dihydro-2*H*-1-benzopyran. 2-Picoline (8.6 g, 93 mmol) was dissolved in THF (200 mL) and cooled to −78 °C. *n*-Butyllithium (50 mL of 2.1 M solution in hexanes, 105 mmol) was added dropwise over 15 min and the solution was stirred for an additional 30 min at −78 °C. A solution of 2-chromanol (14.0 g, 93.0 mmol) and THF (200 mL) was added and the reaction mixture was warmed to room temperature over 2 h and stirred for an additional 2 h. The reaction mixture was then poured onto ice (500 g) and extracted with ethyl acetate (2 × 500 mL). The combined organics were washed with brine (500 mL), dried ($MgSO_4$), and concentrated. The resultant crude yellow solid was recrystallized from hexane/ethyl acetate to afford 1-(2-pyridyl)-4-(2- hydroxyphenyl)-2-butanol (9.4 g, 42%) as yellow plates: mp 115–117 °C; ^{1}H NMR (60 MHz, DMSO-d_6)

δ 1.70 (dt, J = 6, 7 Hz, 2 H), 2.74 (t, J = 7 Hz, 2 H), 2.90 (d, J = 6 Hz, 2 H), 3.92 (app p, J = 6 Hz, 1 H), 4.6 (br s, 1 H), 6.6–7.4 (m, 6 H), 7.49 (dd, J = 2, 8 Hz, 1 H), 8.40 (dd, J = 2, 5 Hz, 1 H).

To a 0 °C solution of 1-(2-pyridyl)-4-(2-hydroxyphenyl)-2-butanol (9.4 g, 39 mmol), triphenylphosphine (10.2 g, 39.0 mmol), and THF (200 mL) was added a solution of diisopropyl azodicarboxylate (7.9 g, 39 mmol) and THF (50 mL) over 20 min and the reaction mixture then stirred at room temperature for 20 h. The reaction was quenched by the addition of water (20 mL) and stirring was continued for an additional 2 h. Methylene chloride (250 mL) was added, and the combined organic layers were washed with water (125 mL), 0.5 M NaOH (50 mL), water (125 mL), and brine (125 mL), and dried ($MgSO_4$). The solvent was removed in vacuo and the residue was purified on silica gel using methylene chloride/methanol (98:2) to afford 2-(2-pyridylmethyl)-3,4-dihydro-2*H*-1-benzopyran (5.1 g, 56%) as a viscous oil: ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.0 (m, 2 H), 2.7 (m, 2 H), 3.08 (dd, J = 3, 6 Hz, 2 H), 4.35 (ddt, J = 3, 6, 12 Hz, 1 H), 6.6–7.3 (m, 6 H), 7.42 (dd, J = 2, 7 Hz, 1 H), 8.25 (dd, J = 2, 5 Hz, 1 H).

To a 0 °C solution of 2-(2-pyridylmethyl)-3,4-dihydro-2*H*-1-benzopyran (1.4 g, 6.3 mmol) and methylene chloride (20 mL) was added titanium tetrachloride (1.4 mL, 12.6 mmol) followed by α,α-dichloromethyl methyl ether (0.63 mL, 7.0 mmol) dropwise over 5 min. The reaction mixture was stirred for 1 h at 0 °C and then slowly poured into cold 10% $NaHCO_3$ (100 mL). The mixture was extracted with methylene chloride (2 × 50 mL), and dthe combined organic layers were washed with water (50 mL) and brine (50 mL), and dried ($MgSO_4$). The solvent was removed in vacuo to afford 760 mg (48%) of a mixture (2/1) of the title aldehyde [^{1}H NMR (60 MHz, $CDCl_3$) δ 9.53, s] along with the 8-formyl isomer [^{1}H NMR (60 MHz, $CDCl_3$) δ 9.95, s].

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Chemical and Biological Studies on a Series of Lipid-Soluble (*trans*-(*R*,*R*)- and -(*S*,*S*)-1,2-Diaminocyclohexane)platinum(II) Complexes Incorporated in Liposomes

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cis-Bis(neodecanoato)(*trans*-(*R*,*R*)-1,2-diaminocyclohexane)platinum(II) [L-NDDP] is a liposome incorporated lipophilic cisplatin analogue that has shown promising antitumor activity against tumors resistant to cisplatin and liver metastases in mice. L-NDDP is currently under clinical evaluation. However, NDDP is an isomeric mixture of different species having various isomeric neodecanoic moities as liganded leaving groups. A series of new highly lipid-soluble *cis*-bis(neodecanoato)(*trans*-(*R*,*R*)- and -(*S*,*S*)-1,2-diaminocyclohexane)platinum(II) [Pt] complexes, using single isomers of neodecanoic acid, were synthesized and characterized by analytical and spectroscopic techniques (infrared and ^{195}Pt NMR). Multilamellar vesicles (MLVs) composed of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG) at a molar ratio of 7:3 were used as carriers of the Pt complexes. The efficiency of incorporation of the liposomal-platinum (L-Pt) preparations was >95% and stability in normal saline at 4 °C was >95% at day 14 in each case. The iv LD_{50} values of all L-Pt preparations tested were in the range of 62.3 to 104 mg/kg. The % T/C obtained after a single ip injection of the optimal dose of L-Pt preparations against L1210 leukemia was in the range of 150 to 253 (160 for cisplatin). When a multiple ip injection schedule was used (on days 1, 5, and 9) the L-Pt preparations of *R*,*R* complexes (1, 7, and 9) were more active than cisplatin at the optimal dose (% T/C = 257 for each vs 220 for cisplatin). The L-Pt preparations of *R*,*R* complexes were also markedly active against L1210 leukemia resistant to cisplatin (% T/C 355, 231, and 185 respectively vs 112 for cisplatin). These studies show that the single isomers of NDDP are comparable to the original isomeric mixture in terms of toxicity and biological activity.

Introduction

Cisplatin is one of the most active antitumor agents.[1,2] The usefulness of cisplatin is, however, compromised by its propensity to cause several severe dose-limiting toxicities including nephrotoxicity, neurotoxicity, and ototoxicity.[3-6] In an attempt to modify the therapeutic index of cisplatin, analogues which are less toxic and non-cross resistant to cisplatin have been synthesized during the last decade. However, the development of some promising analogues has been hampered by their low solubility, formulation problems, and poor stability, which decrease their potential for clinical use.[7]

Another approach to modify the therapeutic index of cisplatin analogues may be the use of drug carriers, among which liposomes are particularly attractive because they are essentially nontoxic, biodegradable lipid vesicles that can alter the distribution and bioavailability of drug.[8,9] The potential use of liposomes as drug carriers has been exploited to improve the therapeutic index of several antimicrobials and anticancer agents. Liposome-incorporated amphotericin B results in an enhancement of the therapeutic index of the drug with lowered toxicity compared to the free drug in the treatment of disseminated candidiasis in both mice[10] and humans.[11] Several investigators

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