Expedited Articles

Discovery of a Potent, Highly Selective, and Orally Efficacious Small-Molecule Activator of the Insulin Receptor

Kun Liu,* Libo Xu, Deborah Szalkowski, Zhihua Li, Victor Ding, Gloria Kwei, Su Huskey, David E. Moller, James V. Heck, Bei B. Zhang, and A. Brian Jones

Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

Received June 29, 2000

A series of 3,6-diaryl-2,5-dihydroxybenzoquinones were synthesized and evaluated for their abilities to selectively activate human insulin receptor tyrosine kinase (IRTK). 2,5-Dihydroxy-6-(1-methylindol-3-yl)-3-phenyl-1,4-benzoquinone (**2h**) was identified as a potent, highly selective, and orally active small-molecule insulin receptor activator. It activated IRTK with an EC_{50} of 300 nM and did not induce the activation of closely related receptors (IGFIR, EGFR, and PDGFR) at concentrations up to 30 000 nM. Oral administration of the compound to hyperglycemic db/db mice (0.1–10 mg/kg/day) elicited substantial to nearly complete correction of hyperglycemia in a dose-dependent manner. In ob/ob mice, the compound (10 mg/kg) caused significant reduction in hyperinsulinemia. A structurally related compound **2c**, inactive in IRTK assay, failed to affect blood glucose level in db/db mice at equivalent exposure levels. Results from additional studies with compound **2h**, aimed at evaluating classical quinone-related phenomena, provided sufficient grounds for optimism to allow more extensive toxicologic evaluation.

Introduction

Insulin is a hormone that is necessary for normal carbohydrate, protein, and fat metabolism in mammals.[1,2] All known actions of insulin are initiated by its binding to the extracellular domain of its specific receptor.[3,4] Following insulin binding, conformational changes in the insulin receptor lead to autophosphorylation of the intracellular β-subunits and stimulation of the receptor's intrinsic tyrosine kinase activity.[5,6] The activated insulin receptor tyrosine kinase (IRTK) phosphorylates several intermediate substrates, which leads to the activation of downstream signaling molecules.[7] Considerable evidence suggests that IRTK activity is essential for many, if not all, of the biological effects of insulin.[1,8] However, the precise biochemical mechanism linking receptor kinase-mediated tyrosine phosphorylation to the regulation of cellular metabolic pathways is not completely defined.

Insulin resistance is a characteristic feature of non-insulin-dependent diabetes mellitus (NIDDM) and is also a contributing factor in atherosclerosis, hypertension, lipid disorders, and polycystic ovarian syndrome.[9,10] NIDDM accounts for more than 90% of diabetes in the United States. This metabolic disorder afflicts an estimated 6% of the adult U.S. population. Conventional treatments for NIDDM have significant limitations. While physical exercise and caloric restriction could improve the diabetic condition, compliance with this treatment is generally poor. Sulfonylureas have been widely used to increase the plasma level of insulin by stimulating the pancreatic β-cells to secrete more insu-

lin. However, dangerously low levels of plasma glucose can result from this treatment.[11] Thiazolidinediones (glitazones) have been recently described as a class of compounds that ameliorates many symptoms of NIDDM.[12–16] These agents substantially increase insulin sensitivity in muscle, liver, and adipose tissue, resulting in the correction of elevated plasma level of glucose without the occurrence of hypoglycemia. However, undesirable effects associated with glitazones have occurred in animal and human studies, including cardiac hypertrophy, hemodilution, and liver toxicity.[17,18] Accordingly, there exists a continuing demand for novel antidiabetic agents.

We recently reported the discovery of an antidiabetic fungal metabolite, **1** (Figure 1).[19] It was isolated from a culture broth of *Pseudomassaria* following an extensive screening effort for small molecules that activate human IRTK. This compound acted as an insulin mimetic in several biochemical and cellular assays. Oral administration of **1** to two mouse models of diabetes resulted in a significant decrease in blood glucose levels. The preliminary studies on **1** demonstrated the feasibility of discovering novel insulin receptor activators that may lead to new therapy for diabetes. However, to support realistic drug development, improvements in potency and selectivity were clearly necessary. It should be noted at the outset that safe, modern quinone-containing drugs do exist. Most notable of these is the antiparasitic Mepron (atovaquone) which has been used in children[20] and even infants[21] and has recently received additional indication for malaria prophylaxis.[22] We herein describe the evaluation of a core set of substituted 2,5-dihydroxyquinones based on the lead compound **1**. 2,5-Dihydroxy-

* To whom correspondence and reprint request should be addressed. Tel: (732) 594 7445. Fax: (732) 594 9556. E-mail: kun_liu@merck.com.
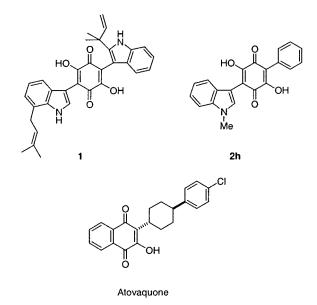
Figure 1. Structures of **1**, **2h**, and atovaquone.

6-(1-methylindol-3-yl)-3-phenyl-1,4-benzoquinone (**2h**) was identified as a potent, highly selective, and orally active antihyperglycemic agent (Figure 1).

Results and Discussion

Chemistry. A series of 3,6-diaryl-2,5-dihydroxybenzoquinones were prepared using the general route previously described for the synthesis of our natural product lead **1** (Scheme 1).[23] The individual compounds **2a–h** described herein are recorded in Table 1.

In Vitro Insulin Receptor Activation and Selectivity. Insulin binds to two asymmetric sites on the α subunits of the insulin receptor and causes conformational changes of the receptor which lead to autophosphorylation of the β subunit and activation of the receptor's intrinsic tyrosine kinase activity.[1-6] A cell-based functional assay using stably transfected Chinese hamster ovary cells expressing human insulin receptors was established and employed as the primary assay for small-molecule insulin receptor activators.[19] In this assay, the ability of test compounds to independently stimulate activation of IRTK was measured. The activities of test compounds were expressed as percentage of control (percent maximal activity achieved with 100 nM insulin). Insulin receptor belongs to a superfamily of receptor tyrosine kinases with high degree of sequence

homology in the tyrosine kinase domain.[4] Thus, to determine the specificity of test compounds for insulin receptor relative to other selected homologous receptors, similar cell-based assays were established and used to counterscreen insulin receptor activators against insulin-like growth factor receptor (IGFIR), epidermal growth factor receptor (EGFR), and platelet-derived growth factor receptor (PDGFR). Maximizing selectivity over such homologous growth factor receptors would clearly be critically important.

Primary in vitro data for a core set of compounds are presented in Table 1. These sketch the deconstructive path taken to define the minimal structural requirement for IRTK activation and some key elements driving selectivity. Data for the previously reported natural product lead **1** is included for comparison. The lead compound **1** activated IRTK with an EC_{50} of 5 μM and weakly activated IGFIR and EGFR at concentrations greater than 30 μM. It was quickly discovered that the prenyl and the reverse prenyl substitutions on the indolyl rings of **1** are superfluous and can be removed without sacrificing in vitro activity on insulin receptor activation. The simplest nonsymmetrical bis-indole dihydroxyquinone **2a** induced 50% of the maximal effect of insulin (100 nM) on IRTK activity at a concentration of 7 μM. Encouraged by this result, **2a** was further simplified by replacing one of the indole moiety with a phenyl ring to give **2b**. Compound **2b** showed a 20-fold increase in IRTK activity with an EC_{50} of 0.3 μM, but it still activated IGFIR and EGFR at higher concentrations. Further simplification to 3,6-bis-phenyl-2,5-dihydroxyquinones resulted in dramatic decrease in potency. Indeed **2c** was inactive in IR activation assay even at a concentration of 100 μM. This demonstrates that the presence of a hydroxyquinone alone is clearly not sufficient for whole-cell insulin receptor activation. Replacement of indole moiety with other classical isosteres such as benzofuran and benzothiophene retained the potency. Compounds **2d,e** had EC_{50}s of 1 and 1.5 μM, respectively. In addition, they showed improved selectivity against IGFIR and EGFR. Both compounds weakly activated IGFIR and EGFR at a concentration of 100 μM, but not at 30 μM. 1-Naphthyl analogue **2f** also retains much of the ability to activate IRTK with an EC_{50} of 6 μM, indicating that the heteroatom is not essential for potency. In contrast, the 2-naphthyl analogue **2g** was some 5 times less potent than **2f**, with an

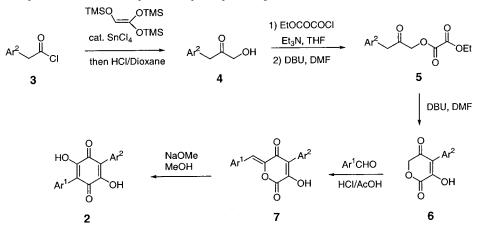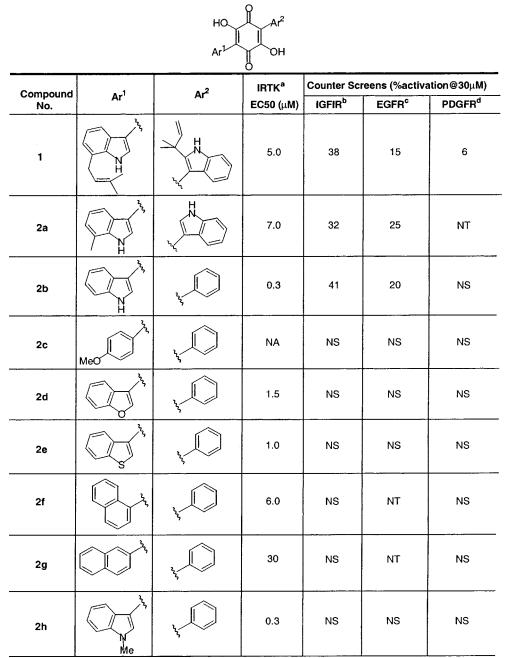**Scheme 1.** General Synthesis of 3,6-Diaryl-2,5-dihydroxybenzoquinones

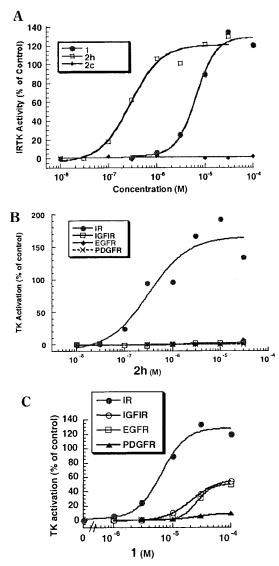**Table 1.** IRTK Assay and Counterscreen Results of 3,6-Diaryl-2,5-dihydroxyquinones

Compound No.	Ar^1	Ar^2	IRTK[a] EC50 (μM)	Counter Screens (%activation@30μM)		
				IGFIR[b]	EGFR[c]	PDGFR[d]
1			5.0	38	15	6
2a			7.0	32	25	NT
2b			0.3	41	20	NS
2c			NA	NS	NS	NS
2d			1.5	NS	NS	NS
2e			1.0	NS	NS	NS
2f			6.0	NS	NT	NS
2g			30	NS	NT	NS
2h			0.3	NS	NS	NS

[a] 50% of maximal activity achieved with 100 nM insulin. [b] Percentage of control (100 nM IGFI). [c] Percentage of control (100 nM EGF). [d] Percentage of control (100 nM PDGF). NA, not active up to 100 μM; NT, not tested; NS, not significant (less than 5% activation).

EC$_{50}$ of 30 μM. This is of note from the point of view of establishing the existence of a precisely defined SAR. The *N*-methyl analogue **2h** was among the most potent insulin receptor activators in this series. It activated the human IRTK with an EC$_{50}$ of 300 nM. More importantly, it did not activate IGFIR, EGFR, and PDGFR at concentrations up to 30 μM.

Some of these data are graphically represented in Figure 2. Two key aspects deserve mention. First, the existence of a spectrum of in vitro IRTK activities among structurally similar hydroxyquinones (Figure 2A) argues against a generic redox activation and provides a critical dyad of compounds (**2h,c**) for validation of the in vivo relevance of the in vitro observation. Second, the dramatic separation of activities at homologous receptors is noteworthy (Figure 2B). For example, IR and

IGFIR are structurally related and highly homologous at the amino acid level,[3,4,24] and clear discrimination of these receptors might, a priori, have been envisaged to be a rather difficult task.

Pharmacokinetics (PK). Pharmacokinetics of compound **2h** in preclinical species are presented in Table 2. In the rat, plasma clearance, volume of distribution at steady state, and terminal half-life ($t_{1/2}$) were 0.04 mL/min/kg, 0.1 L/kg, and 32 h, respectively. After oral dosing at 0.5 mpk, the peak plasma concentration was ~4880 ng/mL, reached 10 h postdose, and oral bioavailability was estimated to be 79%. In dogs and monkeys, the profiles were similar with high C_{max}, high AUC's, slow clearance, and long $t_{1/2}$ values (Table 2). Oral bioavailability remained high in these species at 59% and 67%, respectively.
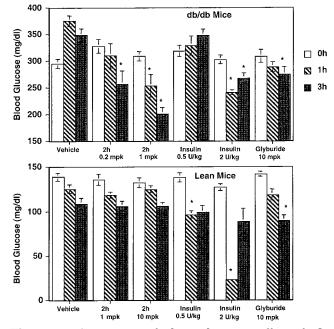
Figure 2. Stimulation of tyrosine kinase activities in CHO cells: (A) dose–response curves for compounds **1** and **2h**,**c** on IRTK activity, (B) selectivity of **2h**, (C) selectivity of **1**.

Table 2. Pharmacokinetic Parameters of **2h** in Preclinical Species

		Intravenous Dose			
species	dose (mg/kg)	AUC (ng·h/mL)	Cl_p (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)
rat	0.05 ($n = 2$)	21770	0.038	0.1	32
dog	0.1 ($n = 2$)	89810	0.019	0.56	93.5
monkey	0.01 ($n = 2$)	8374	0.02	0.31	66.0

		Oral Dose			
species	dose (mg/kg)	$AUC_{0-\infty}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	F (%)
rat	0.5 ($n = 2$)	17600	4879	10	79.3
dog	0.5 ($n = 2$)	265840	5270	2.5	59.2
monkey	0.5 ($n = 2$)	278421	7588	8	66.5

Thus, high and sustained levels of compound are observed, a profile that proved uniform among compounds of this class. It is interesting to note that preclinical and human PK data for other hydroxyquinones are analogous.[25–28]

In Vivo Pharmacology: A. Comparison with Insulin and Glyburide for Acute Efficacy in db/db and Lean Mice. The db/db mouse is an obese
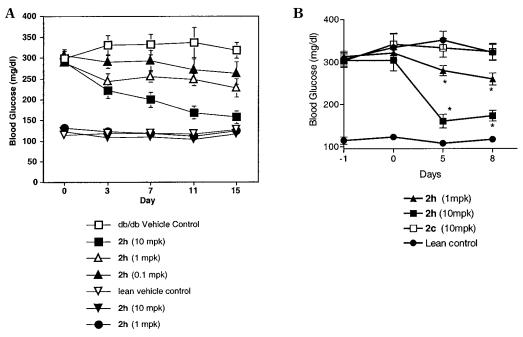

Figure 3. Comparison of glucose-lowering effect of **2h**, insulin, and glyburide in db/db mice or normal lean mice. Food was withheld during the experiment. Blood glucose levels were measured at 1 and 3 h postdosing. *$p < 0.05$, comparing treatment groups with the vehicle control group at the same time point using Student's t-test.

animal model of NIDDM and is characterized by severe insulin resistance and marked hyperglycemia. Under an acute protocol, a single oral dose of compound was administered and blood glucose levels monitored over 24 h. Food was removed during the study to avoid the complicating effect of food intake on blood glucose level. Under these conditions **2h** significantly reduced blood glucose in a dose-dependent manner when monitered at 1–3 h postdosing (35% correction at 0.2 mpk and 50% at 1 mpk) (Figure 3). In a head-to-head experiment, the acute effect of **2h** on hyperglycemia in db/db mice was compared to that of insulin and glyburide (a sulfonylurea insulin secretagogue). The correction of hyperglycemia produced by **2h** is comparable to ip injection of insulin at 2 U/kg. Oral dosing with glyburide at 10 mpk exhibited only a slight glucose-lowering effect.[29] When the same experimental protocol was applied to normal lean, euglycemic mice, **2h** was without effect on glucose levels at 1 and 10 mpk (Figure 3). The lower dose of insulin (0.5 U/kg) produced significant glucose lowering, while severe hypoglycemia was noted at 1 h postinjection of 2 U/kg insulin followed by an expected rebound at 3 h. Oral dosing of glyburide also resulted in a significant glucose-lowering effect.

Of importance is the fact that dihydroxyquinone **2c**, which was inactive in the in vitro IRTK assay, failed to affect hyperglycemia in db/db mice at 30 mpk under the above-mentioned acute protocol.[30] It has been established that compounds **2h**,**c** have very similar pharmacokinetic profiles in rats; therefore, they should have comparable in vivo exposure levels at comparable dosages in the db/db study. These results demonstrated a definitive correlation between in vitro IRTK activity and in vivo glucose-lowering efficacy in animal models.

B. Chronic Efficacy in db/db Mice. Under a chronic dosing protocol, compounds were administered
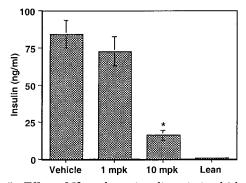
Figure 4. (A) Chronic effect of oral dosing of **2h** on hyperglycemia in db/db mice and lean control mice ($n = 9$/group). Lean control mice and db/db mice were dosed once a day by oral gavage with vehicle or the indicated doses of **2h**. Blood glucose levels were measured 24 h after dosing on the preceding day. p values (one-way ANOVA) for **2h** vs vehicle were: 0.034 (0.1 mpk), <0.001 (1 and 10 mpk). (B) Comparison of chronic effect of **2h**,**c**. *p < 0.01, comparing treatment groups with vehicle control groups ($n = 7-8$/group).

once daily for 8−14 days by gavage to ad libitum fed animals and blood glucose was monitored immediately prior to the next compound dose at days 0, 3, 7, and 11 and at the termination of the study. Such administration of **2h** resulted in significant reduction of blood glucose level in a dose-dependent manner: 29% at 0.1 mpk, 47% at 1 mpk, and 84% at 10 mpk (Figure 4A). No significant effect on blood glucose levels was observed in normal lean mice treated with **2h** at 1 or 10 mpk, and no significant effect on food intake or body weight gain in db/db or lean mice was observed.

To further demonstrate the relevance of in vitro and in vivo observations, the chronic effect of **2h** on hyperglycemia in db/db mice was compared to that of **2c**. The results are displayed in Figure 4B. In this experiment, db/db mice were treated with a daily dosage of **2h**,**c** for 8 days. At day 8, treatment with **2h** resulted in correction of hyperglycemia of 35% at 1 mpk dosage and 76% at 10 mpk dosage. Once again, compound **2c**, which was inactive in the in vitro IRTK assay and had no acute glucose-lowering efficacy in the db/db mice, failed to affect hyperglycemia in db/db mice at 10 mpk in this 8-day chronic study.

Effect on Elevated Insulin Level in ob/ob Mice. The ob/ob mouse is an alternative obese rodent model of insulin resistance that is characterized by extreme hyperinsulinemia. Following single oral dosing of **2h** at 10 mpk, significant decreases in elevated plasma insulin levels were observed 6 h postdosing (food withheld). Further decreases in plasma insulin level were observed 6 h postdosing on day 2 (reaching a net 65% reduction, data not shown). The insulin-lowering effect was sustained at 24 h post-second-dosing (Figure 5). The ob/ob mice were only modestly hyperglycemic at the age tested (12 weeks). Treatment with **2h** (10 mpk) also resulted in a decrease in mean blood glucose concentrations from 233 ± 15 mg/dL (predosing) to 150 ± 6 mg/dL at 24 h

Figure 5. Effect of **2h** on hyperinsulinemia in ob/ob mice ($n = 10$/group). Ob/ob mice were dosed once a day by oral gavage with vehicle or the indicated doses of **2h** for 2 days. The mice were fed ad libitum. Plasma samples were obtained 24 h after dosing on the preceding day and insulin concentrations were measured. *p < 0.00001, compared to the vehicle group.

postdosing ($n = 10$/group). The blood glucose level value for the age- and sex-matched ob/+ lean mice was 143 ± 4 mg/dL ($n = 10$).

Additional Studies. That these compounds are quinones raises inevitable concerns regarding their potential for non-mechanism-based toxicity, though as bis-aryl bis-hydroxy tetrasubstituted quinones, the chemical properties of this particular series would be expected to be rather different from those of unsubstituted ones.[31−37] Quinones are widely distributed in nature and have been isolated from plants, bacteria, and fungi, including many of the foods we eat.[38] Compounds containing the quinone core have long been used in folk medicine[39] and, more recently, as anticancer agents[40] and parasiticides.[41]

The capacity of quinones to redox cycling is perhaps the most obvious pharmaceutical concern.[31,32] Compounds **1** and **2h** did not sustain redox cycling in aqueous DMF as assessed by cyclic voltammetry. How-

ever we had established early in the SAR that the quinone may be reduced by hydrogen sulfide and back-oxidized in air. Among the most obvious targets of aberrant redox activity are interference with the respiratory chain through uncoupling of mitochondrial oxidative phosphorylation,[42] catalysis of the formation of reactive oxygen species,[43] and hemolysis through heme oxidation.[44] In this regard, compound **2h** (10 μM) did not uncouple mitochondrial respiration in rat liver mitochondria (data not shown; *m*-chlorocarbonyl cyanide phenylhydrazone (CCCP) as positive control), was negative in both a non-GLP (good laboratory practice) microbial mutagenesis assay (3–10 000 μg/plate, data not shown) and an in vitro alkaline elution (100–350 μM), and caused no detectable hemolytic anemia in rodents.

Many quinones are also capable of reacting with nucleophiles, in particular thiols.[32] This is clearly a lesser concern with electron-rich, tetrasubstituted quinones.[33] Indeed compound **2h** failed to generate detectable protein modification when incubated with GST-IRTK protein (a glutathione *S*-transferase fusion protein containing the intracellular domain of the insulin receptor) at 5-fold molar excess (data not shown; iodoacetate as positive control).[45]

Conclusion

SAR studies around **1**, an antidiabetic fungal metabolite, resulted in the discovery of 2,5-dihydroxy-6-(1-methylindol-3-yl)-3-phenyl-1,4-benzoquinone (**2h**). This compound is a potent and specific activator of human IRTK in intact mammalian cells. Oral administration of **2h** to diabetic db/db mice elicited substantial correction of elevated levels of blood glucose in a dose-dependent manner. In ob/ob mice, **2h** caused significant decrease in plasma insulin concentration and correction of mild hyperglycemia. Acute or chronic administration of **2h**, up to 10 mpk, did not provoke hypoglycemia in normal mice, which is a very desirable property. A structurally related compound **2c** was inactive in the IRTK assay and also failed to affect blood glucose level in db/db mice at analogous exposure levels. This observation provided a critical validation of the in vivo relevance of the in vitro activity. Despite the concern that the potential value of **2h** may be compromised by features of its chemical structure and its apparently long serum half-life, this compound is chemically well-behaved and demonstrated no overt toxicity at pharmacologic doses in our animal models. It did not poison mitochondrial respiration and did not modify proteins covalently. The discovery of compounds with improved IR activating potency and selectivity as described in this report suggests that future efforts in this field may ultimately lead to a potential new therapy for NIDDM with a mechanism distinct from all other agents currently known.

Experimental Section

All reagents were obtained from commercial suppliers and are used without further purification. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. NMR data were recorded on a Varian XL-400 or Oxford 500 instrument. Chemical shifts are reported in ppm (δ) downfield relative to external TMS (0 ppm) as standard. IR spectra were taken on a Perkin-Elmer 1600 FT-IR spectrometer. Elemental analyses were obtained from Robertson Laboratories (Madison, NJ). All air-sensitive reactions were run under an nitrogen atmosphere.

3,4-Dehydro-3-hydroxy-5-oxo-4-phenyl-δ-valerolactone (6a). To a mixture of phenylacetyl chloride (3.1 g, 20 mmol) and tris(trimethylsilyloxy)ethylene (13.5 g, 44 mmol) at room temperature was added 3 drops of neat TiCl$_4$ via syringe. An exothermic reaction occurred, and the color of the reaction mixture turned to reddish. The reaction mixture was stirred for 3 h before it was poured into a mixture of dioxane (25 mL) and 0.6 N HCl aqueous solution (10 mL). The mixture was stirred at 90 °C for 10 min, cooled to room temperature, and extracted twice with Et$_2$O. The combined organic layers were washed with saturated solution of NaHCO$_3$ and brine, dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo. The residue was crystallized from hexanes to give 2.47 g (82%) of 1-hydroxy-3-phenylpropan-2-one (**4a**) as a white solid: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.35–7.15 (m, 5H, C$_6$H$_5$), 4.26 (d, J = 7.0 Hz, 2H, CH$_2$O), 3.70 (s, 2H, CH$_2$CO), 3.00 (t, J = 7.0 Hz, 1H, OH); MS (CI) *m/e* 151 (M + H).

To a solution of **4a** (2.47 g, 16.4 mmol) in THF (120 mL) at 0 °C was added Et$_3$N (2.7 mL, 19 mmol), followed by ethyl oxalyl chloride (1.9 mL, 17 mmol). The mixture was stirred at 0 °C for 3 h, poured into EtOAc (200 mL), washed with water and brine, dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo to give 3.9 g of ethyl 2,5-dioxo-3-oxy-6-phenylhexanoate (**5a**) as a slightly yellow oil, which was used in the next step without further purification: ^{1}H NMR (CDCl$_3$, 400 MHz) δ 7.35–7.15 (m, 5H, C$_6$H$_5$), 4.85 (s, 2H, CH$_2$O), 4.37 (q, J = 7.0 Hz, 2H, COOCH$_2$), 3.77 (s, 2H, PhCH$_2$CO), 1.38 (t, J = 7.0 Hz, 3H, CH$_3$); MS (CI) *m/e* 251 (M + H).

To a solution of DBU (4.9 mL, 32.8 mL) in DMF (16 mL) at −20 °C was added dropwise a solution of the crude intermediate **5a** from the previous step (3.9 g, 16 mmol) in DMF (16 mL). The reaction mixture was stirred at −15 °C for 2.5 h before it was poured slowly into an ice-cold 1.0 N HCl solution (100 mL). The crystalline product **6a** (1.85 g) was collected by filtration, washed thoroughly with water, and dried under high vacuum. The mother liquid was extracted with EtOAc. The extract was washed with water and brine, dried over MgSO$_4$, filtered and concentrated. The residue was purified by recrystallization from CH$_2$Cl$_2$/hexanes to give another 0.57 g of **6a** as slightly yellow solid. The total yield was 2.42 g (two steps, 72%): mp 174−176 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.50−7.30 (m, 5H, C$_6$H$_5$), 5.11 (s, 2H, OCH$_2$); ^{13}C NMR (acetone-d_6, 400 MHz) δ 189.30, 160.98, 150.96, 130.58, 129.47, 128.53, 127.90, 121.98, 73.39; MS (CI) *m/e* 205 (M + H). Anal. Calcd for C$_{11}$H$_8$O$_4$: C, 64.71; H, 3.95. Found: C, 64.84; H, 3.86.

3,4-Dehydro-3-hydroxy-4-(3-indolyl)-5-oxo-δ-valerolactone (6b). The title compound was prepared following the general procedure described above for the preparation of **6a**: mp 174−176 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.74 (d, J = 2.0 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.13 (t, J = 8.5 Hz, 1H), 7.03 (t, J = 8.5 Hz, 1H), 5.14 (s, 2H); MS (CI) *m/e* 244 (M + H). Anal. Calcd for C$_{13}$H$_9$NO$_4$: C, 64.20; H, 3.73; N, 5.76. Found: C, 64.54; H, 3.96; N, 5.31.

2,5-Dihydroxy-3-(1-methylindol-3-yl)-6-phenyl-1,4-benzoquinone (2h). A mixture of pyrandione **6a** (10.03 g, 49.1 mmol) and 1-methylindole-3-carboxaldehyde (8.0 g, 50.3 mmol) in acetic acid (120 mL) was heated at 60 °C until a clear solution was formed. To this solution was added 2.5 mL of concentrated HCl. The resultant reddish solution was heated at 90 °C for 3 h, during which time red crystalline solid precipitated out. After cooling to room temperature, the reaction was diluted with a 1:1 mixture of ether/hexanes (200 mL), then stirred at 0 °C for 10 min. The precipitate was collected by filtration, washed thoroughly with cold ether/hexanes (1:1) to afford 16.6 g (98%) of 3-hydroxy-6-(1-methylindol-3-ylmethylene)-4-phenyl-2*H*-pyran-2,5(6*H*)-dione (**7h**) as red crystals: ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.16 (s, 1H), 7.95 (d, J = 7.5 Hz, 1H), 7.55 (m, 3H), 7.48 (s, 1H), 7.44 (m, 2H), 7.38 (m, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.27 (t, J = 7.2 Hz, 1H), 4.03 (s, 3H); MS (CI) *m/e* 346 (M + H).

To a suspension of the intermediate obtained above (16.4 g, 47.5 mmol) in methanol (200 mL) at room temperature was

added a solution of NaOMe in MeOH (25 wt %, 60 mL). The mixture was stirred at room temperature for 3 h before it was cooled to 0 °C. 1.5 N HCl aqueous solution (350 mL) was added slowly under stirring. After the addition of HCl was completed, the suspension was stirred at 0 °C for another 15 min. The precipitate was collected by filtration, washed thoroughly with water and hexanes, then dried under high vacuum to give 16.0 g of the crude **2h** as greenish powder. The product was further purified by recrystallization from THF/hexanes to give 15.7 g (96%) of **2h**: mp 267−269 °C; IR (neat) 3303.3, 1631.3, 1333.5, 1234.9 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.6−7.5 (m, 4H), 7.43 (m, 3H), 7.35 (m, 1H), 7.19 (t, $J = 7.2$ Hz, 1H), 7.05 (t, $J = 7.2$ Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 136.89, 132.04, 131.57, 131.20, 128.25, 128.08, 127.46, 122.38, 121.76, 119.52, 115.99, 111.88, 110.36, 104.07, 33.33; MS (CI) m/e 346 (M + H). Anal. Calcd for $C_{21}H_{15}NO_4$: C, 73.03; H, 4.38; N, 4.06. Found: C, 73.11; H, 4.36; N, 4.13.

The following compounds were prepared following the general procedure described above for the preparation of **2h**.

2,5-Dihydroxy-6-(3-indolyl)-3-(7-methylindol-3-yl)-1,4-benzoquinone (2a). The title compound was prepared in 70% yield from **6b** and 7-methylindole-3-carboxaldehyde: mp 172−174 °C; IR (neat) 3328.9, 1629.7, 1341.0 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.40 (s, 1H), 7.69 (d, $J = 3.0$ Hz, 1H), 7.64 (m, 3H), 7.48 (d, $J = 8.0$ Hz), 7.13 (t, $J = 7.2$ Hz, 1H), 7.05 (t, $J = 7.2$ Hz, 1H), 6.95 (m, 2H), 2.52 (s, 3H); HRMS (ESI) m/z calcd for $C_{23}H_{16}N_2O_4$ (M + H) 385.1183, found 385.1193.

2,5-Dihydroxy-3-(3-indolyl)-6-phenyl-1,4-benzoquinone (2b). The title compound was prepared in 85% yield from **6a** and indole-3-carboxaldehyde: mp 237−239 °C; IR (neat) 3312.0, 1630.6, 1340.9, 1232.5 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.66 (d, $J = 2.5$ z, 1H), 7.59 (d, $J = 8.0$ Hz, 1H), 7.56 (d, $J = 8.0$ Hz, 2H), 7.48−7.41 (m, overlapping signals, 3H), 7.36 (m, 1H), 7.14 (t, $J = 7.0$ Hz, 1H), 7.05 (t, $J = 7.0$ Hz, 1H); ^{13}C NMR (acetone-d_6, 400 MHz) δ 136.40, 131.60, 131.21, 128.25, 128.07, 127.08, 122.16, 121.65, 119.35, 115.94, 112.22, 112.12, 104.92; MS (CI) m/e 332 (M + H). Anal. Calcd for $C_{20}H_{13}NO_4$: C, 72.50; H, 3.95; N, 4.23. Found: C, 72.26; H, 4.04; N, 4.21.

2,5-Dihydroxy-3-(4-methoxyphenyl)-6-phenyl-1,4-benzoquinone (2c). The title compound was prepared in 92% yield from **6a** and *p*-anisaldehyde: mp 269−271 °C; ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.40−7.28 (m, overlapping signals, 7H), 7.96 (d, $J = 8.5$ z, 2H), 3.77 (s, 3H); ^{13}C NMR (acetone-d_6, 400 MHz) δ 159.22, 132.38, 131.46, 131.12, 128.25, 128.09, 123.37, 116.13, 116.00, 113.78, 55.79; MS (CI) m/e 323 (M + H). Anal. Calcd for $C_{19}H_{14}O_5$: C, 70.80; H, 4.38. Found: C, 70.84; H, 4.37.

2,5-Dihydroxy-3-(3-benzofuranyl)-6-phenyl-1,4-benzoquinone (2d). The title compound was prepared in 90% yield from **6a** and benzofuran-3-carboxaldehyde: mp 202−204 °C; IR (neat) 3148.7, 1797.0, 1632.5, 1346.6 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.09 (s, 1H), 7.60 (d, $J = 8.4$ Hz, 1H), 7.53 (d, $J = 8.0$ Hz, 1H), 7.42−7.36 (m, overlapping signals, 4H), 7.30 (m, 2H), 7.22 (t, $J = 8.0$ Hz, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 154.82, 146.53, 131.48, 131.16, 128.28, 128.15, 127.35, 124.93, 123.28, 123.15, 116.44, 111.90, 111.33, 108.03; MS (CI) m/e 333 (M + H); HRMS (ESI) m/z calcd for $C_{20}H_{13}O_5$ (M + H) 333.0758, found 333.0762.

2,5-Dihydroxy-3-(3-benzothiopheneyl)-6-phenyl-1,4-benzoquinone (2e). The title compound was prepared in 88% yield from **6a** and benzothiophene-3-carboxaldehyde: mp 251−253 °C; IR (neat) 3285.2, 1630.0, 1343.4 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.99 (d, $J = 7.6$ Hz, 1H), 7.65 (s, 1H), 7.56 (d, $J = 6.4$ Hz, 1H), 7.42−7.38 (m, overlapping signals, 7H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 169.00 (broad), 139.41, 138.92, 131.71, 131.15, 128.36, 128.25, 128.03, 126.90, 124.79, 124.48, 124.44, 123.28, 116.39, 111.11; MS (CI) m/e 349 (M + H). Anal. Calcd for $C_{20}H_{12}O_4S$: C, 68.95; H, 3.47; S, 4.20. Found: C, 68.69; H, 3.39; S, 9.07.

2,5-Dihydroxy-6-phenyl-3-(1-naphthyl)-1,4-benzoquinone (2f). The title compound was prepared in 91% yield from **6a** and 1-naphthaldehyde: mp 149−151 °C; ^{1}H NMR (acetone-

d_6, 400 MHz) δ 7.95 (d, $J = 8.5$ z, 2H), 7.85 (d, $J = 8.2$ Hz, 1H), 7.60−7.55 (m, overlapping signals, 3H), 7.54−7.42 (m, overlapping signals, 5H), 7.36 (m, 1H); ^{13}C NMR (acetone-d_6, 400 MHz) δ 134.00, 132.20, 130.84, 130.75, 128.87, 128.73, 128.48, 128.00, 127.87, 126.19, 126.15, 125.97, 125.49; MS (CI) m/e 343 (M + H). Anal. Calcd for $C_{22}H_{14}O_4$: C, 77.18; H, 4.12. Found: C, 77.06; H, 4.42.

2,5-Dihydroxy-6-phenyl-3-(2-naphthyl)-1,4-benzoquinone (2g). The title compound was prepared in 90% yield from **6a** and 2-naphthaldehyde: mp 287−289 °C; IR (neat) 3318.3, 1611.6, 1320.1 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.08 (s, 1H), 8.00−7.90 (m, overlapping signals, 3H), 7.66 (d, $J = 8.5$ z, 1H), 7.59−7.52 (m, overlapping signals, 4H), 7.44 (t, $J = 8.0$ Hz, 2H), 7.36 (m, 1H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ 133.19, 132.86, 131.50, 131.16, 130.26, 129.16, 129.09, 128.64, 128.29, 128.15, 127.41, 126.97, 126.75, 116.38, 116.23; MS (CI) m/e 343 (M + H). Anal. Calcd for $C_{22}H_{14}O_4$: C, 77.18; H, 4.12. Found: C, 77.06; H, 4.42.

Insulin Receptor (IR) Activation Assay. CHO.IR cells were cultured in 96-well plates at a density of 150 000 cell/well for 24 h and then serum-starved for 2 h prior to treatment with test compounds or insulin for 20 min at 37 °C. The cells were then lysed and cell lysates were added to 96-well poly-(vinyl chloride) plates previously coated with a monoclonal antibody against IR. After incubation for 16 h at 4 °C, the wells were washed three times with TBST. The kinase reaction mixtures were added to each well and the incubation was continued at 25 °C for 40 min. After the addition of phosphoric acid, the reaction mixtures were transferred to multiscreen phosphocellulose plates. The plates were washed with ice-cold phosphoric acid and the radioactivity associated with the filters was determined. In this assay, the EC$_{50}$ for insulin is ~1 nM. The activities of test compounds were expressed as percent (%) of control (maximal activity achieved with 100 nM insulin).

The protocols for IGTIR, EGFR, and PDGFR counterscreens were similar to the one described above for IR.[19]

Determination of Antihyperglycemic Activity of Test Compound in db/db Mice. Male db/db (7−8 weeks of age) and nondiabetic db/+ (lean) mice from Jackson Laboratories were housed 7−9 mice/cage and provided ad libitum access to milled rodent chow (Purina #5008) and water. Food consumption and body weight were monitored during the study. Blood glucose levels were measured using a Lifescan One Touch basic glucometer with small blood samples obtained from the tail. For the acute dosing protocol, 8-week-old mice ($n = 8$/group) were dosed via oral gavage with vehicle (0.5% methylcellulose), compound, glyburide, or via ip injection with vehicle (saline) or insulin. Food was withheld immediately following dosing and blood glucose concentrations were monitored during a 3-h period. For the chronic dosing protocol, mice ($n = 9$/group) received once-a-day oral dosing by gavage with vehicle (0.5% methylcellulose) or compound. The mice were given free access to food and water throughout the study. Blood glucose was monitored immediately prior to the next dose at days 0, 3, 7, 11, and 15. At the termination of the study, blood samples were collected for measurement of hematocrit and serum chemistry determinations.

Determination of Effect on Elevated Insulin Level in ob/ob Mice. Male ob/ob mice (12 weeks of age) and control ob/+ (lean) mice from Jackson Laboratories were housed 7−9 mice/cage and provided ad libitum access to rodent chow (Purina #5008) and water. Food consumption and body weight were monitored during the study. The mice were given daily gavage of vehicle (0.5% methylcellulose) or the indicated doses of compound. Food was removed 0−6 h postdosing and reintroduced thereafter on days 1 and 2. Free access to water was allowed throughout the study. Plasma samples were collected at 6 h postdosing on days 1 and 2 (food withheld) or 24 h postdosing on day 3 (fed ad libitum). The plasma sample were frozen and analysis of insulin was performed using a commercial RIA kit.

Pharmacokinetic Studies. Male rats, beagle dogs and monkeys (rhesus macaque) were dosed iv or orally with compound. The iv doses were prepared in ethanol/PEG400/

water (1:3:6, v/v/v). The oral doses for dog and monkey studies were prepared in 0.5% methylcellulose suspension containing 0.02% sodium lauryl sulfate; the oral dose for rat study was in ethanol/PEG400/water (1:3:6, v/v/v). Plasma samples were extracted and the concentrations of **2h** in plasma were determined by LC−MS/MS (LOQ = 8−100 ng/mL) with 2,5-dihydroxy-6-(4-methoxyphenyl)-3-(1-methylindol-3-yl)-1,4-benzoquinone as the internal standard.

References

(1) Rosen, O. M. Structure and Function of Insulin Receptors. *Diabetes* **1989**, *38*, 1508−1511.
(2) Rosen, O. M. After Insulin Binds. *Science* **1987**, *237*, 1452−1458
(3) Ebina, Y.; Ellis, L.; Jarnagin, K.; Edery, M.; Graf, L.; Clauser, E.; Ou, J.; Masiarz, F.; Kan, Y. W.; Goldfine, I. D.; Roth, R. A.; Rutter, W. J. The Human Insulin Receptor cDNA: The structural Basis for Hormone-Activated Transmenbrane Signaling. *Cell* **1985**, *40*, 747−758.
(4) Ullrich, A.; Bell, J. R.; Chen, E. Y.; Herrera, R.; Petruzzelli, L. M.; Dull, T. J.; Gray, A.; Coussens, L.; Liao, Y. C.; Tsubokawa, M.; Mason, A.; Seeburg, P. H.; Grunfeld, C.; Rosen, O. M.; Ramachandran, J. Human Insulin Receptor and Its Relationship to the Tyrosine Kinase Family of Oncogenes. *Nature* **1985**, *313*, 756−762.
(5) Hubbard, S. R.; Wei, L.; Ellis, L.; Hendrickson, W. A. Crystal Structure of the Tyrosine Kinase Domain of the Human Insulin Receptor. *Nature* **1994**, *372*, 746−754.
(6) Hubbard, S. R. Crystal Structure of the Activated Insulin Receptor Tyrosine Kinase in Complex with Peptide Substrate and ATP Analogue. *EMBO J.* **1997**, *16*, 5572−5581.
(7) White, M. F.; Kahn, C. R. In *Insulin Resistance*; Moler, D. E., Ed.; Wiley: Chichester, U.K., 1994; pp 9−24.
(8) Russell, D. S.; Gherzi, R.; Johnson, E. L.; Chou, C.-K.; Rosen, O. M. The Protein Tyrosine Kinase Activity of the Insulin Receptor Is Necessary for Insulin-mediated Receptor Downregulation. *J. Biol. Chem.* **1987**, *262*, 11833−11840.
(9) Defronzo, R. A.; Bonadonna, R. C.; Ferrannini, E. Pathogenesis of NIDDM. *Diabetes Care* **1992**, *15*, 318−368.
(10) Reaven, G. Role of Insulin Resistance in Human Disease. *Diabetes* **1998**, *37*, 1595−1607.
(11) Holman, R. R.; Turner, R. C. Oral Agents and Insulin in the Treatment of Non-Insulin Dependent Diabetes Mellitus. In *Textbook of Diabetes*; Pickup, J. C., Williams, G., Eds.; Blackwell Scientific Publications: London, 1991; pp 462−476.
(12) Fujiwara, T.; Yoshioka, S.; Yoshioka, T.; Ushiyama, I.; Horikoshi, H. Charaterization of New Oral Antidiabetic Agent CS-045. *Diabetes* **1988**, *37*, 1549−1558.
(13) 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 Thiazolidine-2,4-diones as Potent Euglycemic Agents. *J. Med. Chem.* **1992**, *35*, 1853−1864.
(14) Cantello, B. C.; Cawthorne, M. A.; Haigh, D.; Hindley, R. M.; Smith, S. A.; Thurlby, P. L. The Synthesis of BRL-49653 − A Novel and Potent Antihyperglycemic Agent. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1181−1184.
(15) Hulin, B.; McCarthy, P. A.; Gibbs, E. M. The Glitazone Family of Antidiabetic Agents. *Curr. Pharm. Des.* **1996**, *2*, 85−102.
(16) Reddy, K. A.; Hohray, B. B.; Bhushan, V.; Bajji, A. C.; Reddy, K. V.; Reddy, P. R.; Krishna, T. H.; Rao, I. N.; Jajoo, H. K.; Rao, N. V. S. M.; Chakrabarti, R. Novel Antidiabetic and Hypolipidemic Agents. 3. Benzofuran-Containing Thiazolidinediones. *J. Med. Chem.* **1999**, *42*, 1927−1940.
(17) Ishii, S.; Wasaki, M.; Ohe, T.; Ueno, H.; Tanaka, H. MCC-555: A Highly Potent Thiazolidinedione Lacking Hematological and Cardiac Side-effects. *Diabetes* **1996**, *45* (Suppl. 2), 141A (abstract).
(18) Watkins, P. B.; Whitecomb, R. W. Hepatic Dysfunction Associated with Troglitazone. *N. Engl. J. Med.* **1998**, *338*, 916−917.
(19) Zhang, B.; Salituro, G.; Szalkowski, D.; Li, Z.; Zhang, Y.; Royo, I.; Vilella, D.; Diez, M. T.; Pelaez, F.; Ruby, C.; Kendall, R. L.; Mao, X.; Griffin, P.; Calaycay, J.; Zierath, J. R.; Heck, J. V.; Smith, R. G.; Moller, D. E. Discovery of a Small Molecule Insulin Mimetic with Antidiabetic Activity in Mice. *Science* **1999**, *284*, 974−977.
(20) Sabchareon, A.; Attanath, P.; Phanuaksook, P.; Chanthavanich, P.; Poonpanich, Y.; Mookmanee, D.; Chongsuphajaisiddhi, T.; Sadler, B. M.; Hussein, Z.; Canfield, C. J.; Hutchinson, D. B. Efficacy and Pharmacokinetics of Atovaquone and Proguanil in Children with Multidrug-resistant Plasmodium falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg.* **1998**, *92*, 201−206.
(21) Hughes, W.; Dorenbaum, A.; Yogev, R.; Beauchamp, B.; Xu, J.; McNamara, J.; Moye, J.; Purdue, L.; van Dyke, R.; Rogers, M.; Sadler, B. Phase I Safety and Pharmacokinetics Study of Micronized Atovaquone in Human Immunodeficiency Virus-infected Infants and Children. *Antimicrob. Agents Chemother* **1998**, *42*, 1315−1318.
(22) Lell, B.; Luckner, D.; Ndjave, M.; Scott, T.; Kremsner, P. G. Randomised Placebo-controlled Study of Atovaquone Plus Proguanil for Malaria Prophylaxis in Children. *Lancet* **1998**, *351*, 709−713.
(23) Liu, K.; Wood, H. B.; Jones, A. B. Total Synthesis of Asterriquinone B1. *Tetrahedron Lett.* **1999**, *40*, 5119−5122.
(24) Massague, J.; Czech, M. P. The Subunit Structures of Two Distinct Receptors for Insulin-like Growth Factors I and II and Their Relationship to the Insulin Receptor. *J. Biol. Chem.* **1982**, *257*, 5038−5045.
(25) Hughes, W. T.; Gray, V. L.; Gutteridge, W. E.; Latter, V. S.; Pudney, M. Efficacy of a Hydroxynaphthoquinone, 566C80, in Experimental *Pneumocystis carinii* Pneumonitis. *Antimicrob. Agents Chemother.* **1990**, *34*, 225−228.
(26) Rolan, P. E.; Mercer, A. J.; Weatherley, B. C.; Holdich, T.; Merie, H.; Peck, R. W.; Ridout, G.; Posner, J. Examination of Some Factors Responsible for a Food-induced Increase in Absorption of Atovaquone. *Br. J. Clin. Pharmacol.* **1994**, *37*, 13−20.
(27) Dixon, R.; Pozniak, A. L.; Watt, H. M.; Rolan, P.; Posner, J. Single-dose and Steady-State Pharmacokinetics of a Novel Microfluidized Suspension of Atovaquone in Human Immunodeficiency Virus-Seropositive Patients. *Antimicrob. Agents Chemother.* **1996**, *40*, 556−560.
(28) Hussein, Z.; Eaves, J.; Hutchinson, D. B.; Canfield, C. J. Population Pharmacokinetics of Atovaquone in Patients with Acute Malaria Caysed by *Plasmodium falciparum*. *Clin. Pharmacol. Ther.* **1997**, *61*, 518−530.
(29) In experiments not shown here, thiazolidinedione insulin sensitizers had no effect on glucose in the acute db/db protocol described above, suggesting different mechanisms of action for thiazolidinediones and **2h**.
(30) Zhang, B.; et al. Manuscript submitted.
(31) O'Brien, P. J. Molecular Mechanism of Quinone Cytotoxicity. *Chen.-Biol. Interact.* **1991**, *80*, 1−41.
(32) Gant, T. W.; Rao, D. N. R.; Mason, R. P.; Cohen, G. M. Redox Cycling and Sulphydryl Arylation: Their Relative Importance in the Mechanism of Quinone Cytotoxicity to Isolated Hepatocytes. *Chem.-Biol. Interact.* **1988**, *65*, 157−173.
(33) Finley, K. T. The Addition and Substitution Chemistry of Quinones. In *The Chemistry of the Quinonoid Compounds, Part II*; Patai, S., Ed.; John Willey: London, 1974; p 878.
(34) Nickerson, W. J.; Falcone, G, Strass, G. Studies on Quinone Thioesters. I. Mechanism of Formation and Properties of Menadione. *Biochemistry* **1963**, *2*, 537−543.
(35) Ross, D.; Thor, S.; Orrenius, S.; Moldeus, P. Interaction of Menadione with Glutathione. *Chem.-Biol. Interact.* **1985**, *55*, 177−184.
(36) Miller, M. G.; Rodgers, A.; Cohen, G. M. Mechanisms of Toxicity of Naphthoquinones to Isolated Hepatocytes. *Biochem. Pharmacol.* **1986**, *35*, 1177−1184.
(37) Ross, D.; Thor, H.; Threadgill, M. D.; Sandy, M. S.; Smith, M. T.; Moldeus, P.; Orrenius, S. The Role of Oxidative Processes in the Cytotoxicity of Substituted 1,4-Naphthoquinones in Isolated Hepatocytes. *Arch. Biochem. Biophys.* **1986**, *248*, 460−466.
(38) Thomson, R. H. *Naturally Occurring Quinones. III: Recent Advances*; Chapman and Hall: London, 1987.
(39) Duke, J. A. *Handbook of Medicinal Herbs*; CRC Press: Boca Raton, FL, 1985.
(40) Powis, G. Metabolism and Reactions of Quinoid Anti-cancer Agents. *Pharmacol. Ther.* **1987**, *35*, 57−162.
(41) Hudson, A. T. Atovaquone-A Novel Broad-Spectrum Anti-Infective Drug. *Parasitol. Today* **1993**, *9*, 66−68.
(42) De Hann, E. J.; Charles, R. The Mechanism of Uncoupling of Oxidative Phosphorylation by 2-Methyl-1,4-naphthoquinone. *Biochim. Biophys. Acta* **1969**, *180*, 417−419.
(43) Chesis, P. L.; Levin, D. E.; Smith, M. T.; Ernster, L.; Ames, B. N. Mutagenicity of Quinones: Pathways of Metabolic Activation and Detoxification. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1696−1700.
(44) Munday, R.; Smith, B. L.; Fowke, E. A. Haemolytic Activity and Nephrotoxicity of 2-Hydroxy-1,4-naphthoquinone in Rats. *J. Appl. Toxicol.* **1991**, *11*, 85−90.
(45) The natural product lead **1** was subsequently demonstrated to activate TrkA, -B and -C in the low micromolar range using CHO cells stably expressing human Trk receptors. However, compound **2h** was found to have no effect at similar concentrations (N. Wilkie et al., manuscript submitted).