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Development of Novel Alkene Oxindole Derivatives As Orally Efficacious AMP-Activated Protein Kinase Activators

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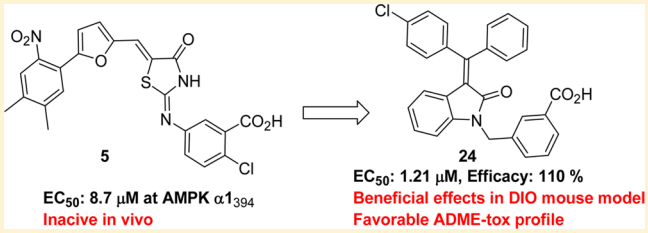
Supporting Information

ABSTRACT: Adenosine 5′-monophosphate-activated protein kinase (AMPK) is emerging as a promising drug target for its regulatory function in both glucose and lipid metabolism. Compound PT1 (**5**) was originally identified from high throughput screening as a small molecule activator of AMPK through the antagonization of the autoinhibition in α subunits. In order to enhance its potency at AMPK and bioavailability, structure−activity relationship studies have been performed and resulted in a novel series of AMPK activators based on an alkene oxindole scaffold. Following their evaluation in pharmacological AMPK activation assays, lead compound **24** was identified to possess improved potency as well as favorable pharmacokinetic profile. In the diet-induced obesity (DIO) mouse model, compound **24** was found to improve glucose tolerance and alleviate insulin resistance. The in vitro and in vivo data for these alkene oxindoles warrant further studies for their potential therapeutic medications in metabolic associated diseases.



KEYWORDS: *AMPK activator, alkene oxindole, DIO mouse model, insulin sensitivity, diabetes*

Adenosine 5′-monophosphate-activated protein kinase (AMPK) is an evolutionarily conserved serine/threonine protein kinase, which exists as a heterotrimer comprising a catalytic α-subunit and two regulatory β and γ subunits. Different combinations of the three subunits encoded by seven genes (α1, α2; β1, β2; γ1, γ2, and γ3) result in different $\alpha\beta\gamma$ heterotrimers. The α2 subunit is highly abundant in skeletal muscle, heart, and liver, while AMPK α1 subunit is relatively evenly distributed across rat heart, liver, kidney, brain, spleen, lung, and skeletal muscle.[1] The β1 and γ1 subunits are expressed universally, while β2, γ2, and γ3 subunits are relatively more confined to muscle or heart. AMPK acts as a cellular energy sensor and regulator, balancing the energy at both the cellular level and the whole body. In mammals, the activity of AMPK is regulated by predominant upstream kinases liver kinase B1 (LKB1) and calcium/calmodulin-dependent protein kinase kinase (CaMKKα/β) through the phosphorylation of a conserved threonine (Thr 172 in rat).[2,3] Once activated by hypoxia, pressure overload, or hypertrophy, AMPK recovers intracellular energy balance either by stimulating the activity of catabolic pathways that generate ATP, such as fatty acid oxidation, or inhibiting the activity of ATP-consuming anabolic pathways, such as the synthesis of fatty acids, cholesterol, glycogen, and proteins.[4] Recent reports have demonstrated that AMPK activation plays a central role in the regulation of body weight, systemic glucose homeostasis, lipid metabolism, and mitochondrial biogenesis. The two endogenous adipokines, leptin and adiponectin exhibit beneficial effects on glucose and lipid metabolism by activating AMPK.[5−7] A number of pharmacological agents including two major classes of existing antidiabetic drugs biguanides and thiazolidinediones were found to exert their effects at least partially by activating AMPK.[8,9] Therefore, activators of AMPK represent a promising therapeutic approach for the treatment of type 2 diabetes and metabolic syndromes.

5-Aminoimidazole-4-carboxamide riboside (AICAR) (Figure 1) has been widely used as an AMPK activator via conversion into 5-amino-4-imidazolecarboxamide ribotide (ZMP) by

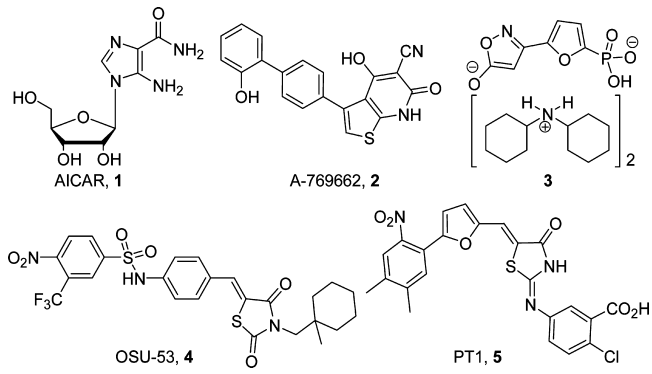


Figure 1. Structures of selected AMPK activators.

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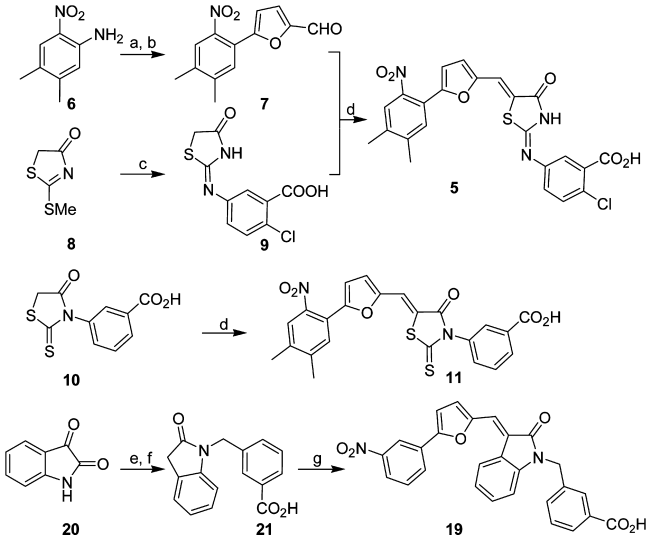
adenosine kinase inside the cell to regulate cellular metabolism. Currently, pharmaceutical companies and academic bodies are actively involved in the discovery and development of small-molecule AMPK activators. Four research groups have published AMPK activators, which were originally identified in enzymatic assays and exhibited promising in vitro and/or in vivo activities. The thienopyridone A-769662 (**2**) identified by Abbott Laboratories was the first reported compound to stimulate partially purified rat liver AMPK (EC_{50} = 0.8 μM) and inhibit fatty acid synthesis in primary rat hepatocytes (IC_{50} = 3.2 μM).[10] Treatment of *ob/ob* mice with compound **2** decreased the plasma glucose, body weight gain, and both the plasma and liver triglyceride levels. 5-(5-Hydroxy-isoxazol-3-yl)-furan-2-phosphonic acid (**3**) was identified by Metabasis Therapeutics as a tool compound for AMPK activator through the screening of a library of AMP mimetics that showed high potency for AMPK (EC_{50} = 6 nM vs AMP EC_{50} = 6 μM).[11] Compound **4** was found to stimulate recombinant AMPK activity (EC_{50} = 0.3 μM) and showed translational potential for breast cancer therapy.[12] From our own research institute, compound PT1 (**5**) was originally identified as a novel small molecule that activates AMPK through antagonizing the autoinhibition in α subunits.[13] It activated the inactive forms of AMPK $\alpha2_{398}$ (α2, residues 1−398) and $\alpha1_{394}$ (α1, residues 1−394) with moderate activity (EC_{50} = 12 and 9 μM). Compound **5** was found to stimulate the phosphorylation of AMPK and acetyl-CoA carboxylase (ACC) and dose-dependently decreased the lipid content in HepG2 cells. However, compound **5** was not active in vivo (unpublished data), probably due to the insufficient potency and poor bioavailability. Here, we present our efforts in the optimization of this reported hit compound **5** that led to the identification of alkene oxindole derivatives as novel AMPK activators that activates AMPK in vitro and more importantly showed beneficial metabolic effects on diet induced obese (DIO) mouse model.

Following the discovery of hit compound **5** from our library screening against AMPK, we devised a synthetic route for this compound in order to confirm its activity (Scheme 1). Initially, Meerwein arylation of 4,5-dimethyl-2-nitro-phenylamine **6** with furan-2-carboxylic acid afforded the acid, which was subsequently reduced with borane-THF and then oxidized with manganese dioxide to furnish the aldehyde **7**. Methylrhodanine **8** was reacted with 5-amino-2-chloro-benzoic acid to afford the thiazolidinone **9**, which in turn was condensed with the aldehyde **7** to afford compound **5**. Utilizing this synthetic route, compounds **12**−**18** were prepared starting from different aminobenzenes as shown in Table 1. The synthesized compounds were initially characterized using an inactive $\alpha1_{394}$ form to measure AMPK activation under typical assay conditions containing 2 μL of compound (20 μg/mL) dissolved in dimethyl sulfoxide, in which compound **5** reached the maximum activation.

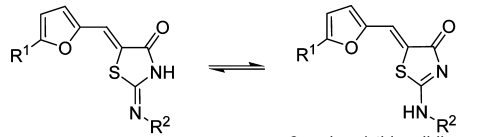
The overlapping broad signals in the ^{1}H NMR spectra of 2-imino-4-thiazolidinones **5** and **12**−**18** are consistent with the previous reports that these compounds exist either as amino or the imino tautomer in the solution state.[14−17] In order to eliminate this tautomerization, a synthetic route was devised from the known compound **10** to access compound **11** (Scheme 1). The activity dramatically decreased when compared to the original compound **5** (Table 1). In the subsequent structure−activity relationships (SAR) studies, the replacement of the central 2-imino-4-thiazolidinone with 3-alkylideneoxindole ring system was attempted since it

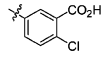
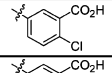
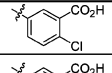
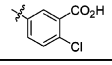
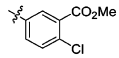
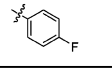
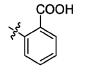
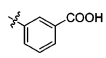
Scheme 1[a]



[a]Reagents and conditions: (a) i. $NaNO_2$, 4 N aq. HCl; ii. furan-2-carboxylic acid, CuCl; (b) i. BH_3·THF, THF; ii. MnO_2, $CHCl_3$; (c) EtOH, reflux; (d) **7**, NaOAc, AcOH, reflux; (e) 3-bromomethyl-benzoic acid methyl ester, NaH, DMF; (f) i. LiOH, dioxane, H_2O; ii. N_2H_4.H_2O, reflux; (g) 3-nitro-phenyl-furan-2-carbaldehyde, pyridine, EtOH, reflux.

Table 1. Initial measurement of AMPK Activation Using Inactive $\alpha1_{394}$ Form[a]



ID	R^1	R^2	Activation[b]
5[c]			100 %
12			< 50 %
13			< 50 %
14			176 %
15			< 50 %
16			< 50 %
17			55 %
18			106 %
11	-	-	< 50 %
19[c]	-	-	341 %

[a]See Experimental Section, Supporting Information. [b]Activation relative to compound **5**, 20 μg/mL. [c]The EC_{50} values of **5** and **19** were determined (Supporting Information Figure 1): **5**, EC_{50} = 9.6 μM; **19**, EC_{50} = 2.1 μM.

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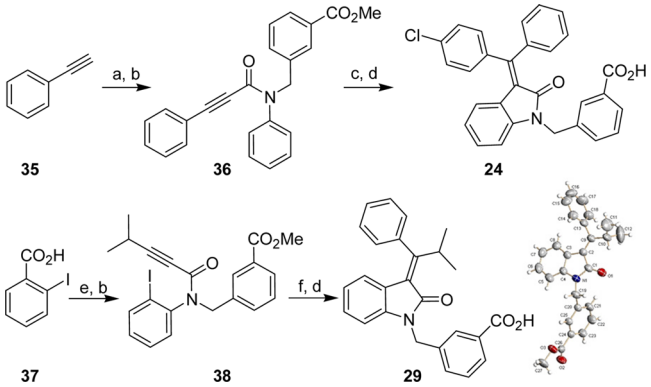
represents an important substructure found in naturally occurring bioactive indole alkaloids. Accordingly, 3-alkylideneoxindole **19** was designed and synthesized as shown in Scheme 2. *N*-Alkylation of isatin **20** with 3-bromomethyl-

Scheme 2[a]

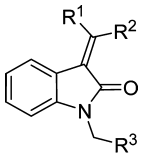


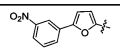
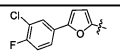
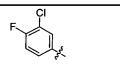
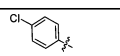
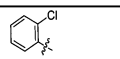
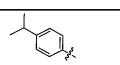
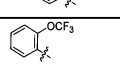
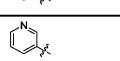
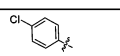
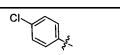
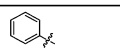
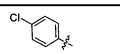
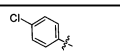
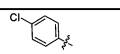
[a]Reagents and conditions: (a) phenyl isocyanate, EtMgBr; (b) 3-bromomethyl-benzoic acid methyl ester, Cs_2CO_3, $(^nBu)_4N^+I^-$, DMF; (c) ArI, cat. $Pd(OAc)_2$, NaOAc, DMF; (d) LiOH, 1,4-dioxane, or THF, H_2O; (e) i. DPPA, Et_3N, PhH; ii. 1,1-dibromo-3-methyl-but-1-ene, ^nBuLi, THF; (f) 4-chlorobenzeneboronic acid, $Pd(OAc)_2$, PPh_3, CsF, THF.

benzoic acid methyl ester afforded the intermediary ester. Hydrolysis of methyl ester and reduction of carbonyl group with hydrazine hydrate provided the oxindole. Aldol condensation of **21** with 3-nitro-phenyl-furan-2-carbaldehyde afforded **19**. Gratifyingly, compound **19** increased the AMPK activation by 3-fold at the initial tested dose of 20 μg/mL (Table 1) compared to compound **5**. A dose−response curve was then calculated for **19** (see Supporting Information), where it was shown that this compound activated the recombinant AMPK $\alpha1_{394}$ with an EC_{50} value of 2.1 μM. The potency of **19** in the activation of AMPK $\alpha1_{394}$ is improved about 5-fold compared to that of compound **5** (EC_{50} = 9.6 μM). As nitroaromatic moiety is often associated with toxicity via producing the potentially hazardous nitroanion radical, nitroso intermediate, and *N*-hydroxy derivative in medicinal chemistry,[16−18] replacement with halogens was attempted as exemplified by 3-chloro-4-fluoro substitution. In a similar manner, compound **20** was synthesized using 3-chloro-4-fluorophenyl-furan-2-carbaldehyde. The two novel oxindoles **19** and **20** were tested using a scintillation proximity assay for their ability to activate the bacterial expressed isoform of AMPK $\alpha2\beta1\gamma1$ as described in Table 2. Both of them exhibited enhanced potency and efficacy compared to compound **5**. Furthermore, it was found that the potency and efficacy were retained with the biaryl substitutions of the alkene oxindole (**23**, EC_{50} = 5.9 μM, efficacy 105%) compared to the diaryls (**22**, EC_{50} = 4.8 μM, efficacy 122%).

Encouraged by the improvement of the AMPK activation of alkene oxindoles compared to the thiazolidinones, a subsequent broader range of structural modifications were carried out. The SAR studies were mainly focused on the 3 positions (Table 2; R^1, R^2, and R^3). Compounds **23**, **25**−**28**, and **32**−**34** were synthesized utilizing the same synthetic routes as described for **24** shown in Scheme 2. The synthetic approach was based on a highly efficient palladium-catalyzed synthesis of asymmetrically substituted alkene oxindoles from readily accessible starting

Table 2. Activation of the AMPK Heterotrimer $\alpha2\beta1\gamma1$.[a]



ID	R^1	R^2	R^3	EC_{50} μM	Efficacy[b]
5	-	-	-	0.3	40 %
19		H		3.2	152 %
22		H		4.8	122 %
23				5.9	105 %
24				1.2	110 %
25				1.8	101 %
26				4.0	97 %
27				7.4	99 %
28				3.2	96 %
29				9.9	227 %
30				4.6	103 %
31				NA[b]	NA
32				9.2	75 %
33				4.5	151 %
34				1.0	70 %

[a]See Experimental Section, Supporting Information. [b]Efficacy relative to AMP. [c]Not active, defined as EC_{50} > 10 μM or efficacy < 50% activation of AMP.

materials.[19] Initially, commercially available ethynyl benzene was reacted with phenyl isocyanate in the presence of ethyl magnesium bromide to form the unsaturated amide. This intermediate was subsequently reacted with 3-bromomethyl-benzoic acid methyl ester using cesium carbonate as a base and catalytic amount of *n*-tetrabutylammonium iodide. Palladium-(II) acetate-catalyzed coupling with appropriate aryl iodides using *N*,*N*-dimethylformamide as a solvent and NaOAc as a base at 110 °C afforded the key alkene oxindoles. Hydrolysis of an ester group gave the desired final compounds. Compounds **29**−**31** were synthesized utilizing the synthetic routes shown in Scheme 2, exemplified by compound **29**. Curtius rearrangement of 2-iodobenzoic acid using diphenylphosphoryl azide and triethylamine in benzene afforded the intermediate isocyanate. 1,1-Dibromo-3-methyl-but-1-ene was reacted with *n*-butyl lithium under Corey−Fuchs condition to give the intermediary alkynyl anion in situ, which was then coupled with the isocyanate to give the unsaturated amide. This intermediate was subsequently reacted with 3-bromomethyl-benzoic acid methyl ester using cesium carbonate as a base and catalytic

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amount of *n*-tetrabutylammonium iodide. The key palladium-catalyzed Heck-carbocyclization/Suzuki-coupling reaction between iodide **38** and benzeneboronic acid in the presence of catalytic amount of $Pd(PPh_3)_4$ afforded the alkene oxindole, followed by ester hydrolysis. This reaction proceeded smoothly when CsF or copper thiophene-2-carboxylic acid was used as a base.[20,21] The configuration of this series of compounds was confirmed by the crystal structure (methyl ester of compound **29**) as shown in Scheme 2.

The alkene oxindole derivatives were screened for their ability to activate the AMPK heterotrimer $\alpha2\beta1\gamma1$, and the EC_{50} and efficacy values were obtained (Table 2). Different electron withdrawing and donating groups were selected for modification at R^1 position. The R^2 and R^3 were maintained throughout the series as a phenyl and benzyl-3-carboxylic acid, respectively. For the R^1 position, a 2- or 4-chloro substitution enhanced the potency (EC_{50} = 1.8 and 1.2 μM) compared to 3-chloro-4-fluoro substituted **23** (EC_{50} = 5.9 μM). The 3-pyridyl group (**28**) also retained the activity with an EC_{50} value of 3.2 μM and efficacy value of 96%. When 4-chloro substitution at the R^1 position was deleted, the AMPK activity was abolished (**31**). Maintaining R^1 position as a 4-chlorophenyl and R^3 as a benzyl-3-carboxylic acid, some structural variations were explored at the R^2 position. A cyclohexyl group at the R^2 position (**30**) was found to retain the efficacy but with a 4-fold decrease in potency compared to **24**. An isopropyl group (**29**) resulted in about 10-fold lower potency (EC_{50} = 9.9 μM) and 2-fold higher efficacy compared to **24**. Deletion of the carboxylic acid moiety at the benzyl ring (**34**) resulted in an approximately 40% decrease in the efficacy compared to **24**. Substitution of the benzyl-3-carboxylic acid (**24**) with benzyl-3-carboxamide (**33**) resulted in a slight decrease of the potency (1.2 vs 4.5 μM) but accompanied with about 50% increase of efficacy. Exchanging the phenyl central core at R^3 by a pyridinyl (**32**) led to the decrease of both the potency and efficacy.

After observing the activation of AMPK on a molecular level, its effect in cells was next investigated in L6 myoblasts. The phosphorylation of AMPK and its downstream substrate ACC was monitored as markers for the activation status of AMPK pathways. Western blotting analysis (Figure 2a) showed that compound **19**, **22**, and **24** activated the cellular AMPK activity in a dose-dependent manner, and this effect was confirmed by the dose-dependently increased phosphorylation of ACC. Because the activation of AMPK in muscle has been linked to the stimulation of glucose uptake, we next assessed this effect (Figure 2b). Treatment of L6 myotubes with **24** for 3 h

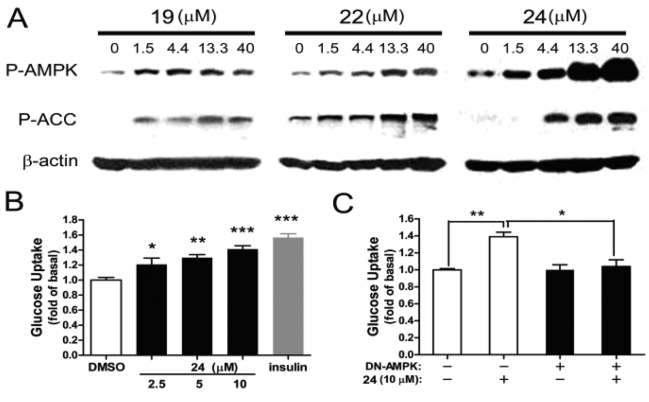

Figure 2. Efficacy on cultured L6 myotubes.

stimulated glucose uptake dose-dependently, which was blocked in the dominant-negative mutant of AMPK (DN-AMPK). The results are presented as the mean ± SE: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ (n = 3 replicated assays). Additionally, no significant off-target effects of **24** were observed toward two closely related kinases in the AMPK family: MARK2 and MELK, as well as Aurora A, GSK3β, and IKKβ.

In vitro metabolism studies of compounds **24** showed no detectable metabolism at both mouse and rat liver microsomes. The hepatic clearance rate of **24** was 8.6 mL/min/kg in human liver microsomes. Assays of the compound's inhibitory potential toward multiple CYP450 enzymes including CYP3A4, CYP2C9, CYP2D6, CYP2C19, and CYP1A2, revealed no significant inhibition, suggesting that **24** will not alter the metabolism of other exnobiotics or endogenous compounds that are substrates for the CYP isoforms tested. Cardiotoxicity associated with the inhibition of human ether-a-go-go-related gene was also evaluated, where no obvious inhibition was seen (IC_{50} > 20 μM). To further explore the bioavailability of alkene oxindole series of activators, compound **24** was selected for full in vivo pharmacokinetic studies on Wistar rats. Compound was intravenously and orally administered to rats with the dose of 2 and 10 mg/kg, respectively. The plasma samples were collected after dosing, and the drug concentrations in plasma were determined by a developed LC–MS/MS method. After an intravenous dose of 2 mg/kg, the mean terminal half-life ($t_{1/2}$) value of **24** was 3.94 h, and the mean values of $AUC_{0-24\ h}$ and $AUC_{0-\infty}$ were about 17.3 and 17.5 μg·h/mL (Supporting Information Table 1 and Figure 2). After an oral dose of 10 mg/kg of **24**, plasma concentrations in rats reached peaks with a mean value of 3.27 μg/mL at 4.0 h post dose. The mean $t_{1/2}$ value was about 4 h. The mean values of $AUC_{0-24\ h}$ and $AUC_{0-\infty}$ of **24** were 28.9 and 29.5 μg·h/mL, respectively. On the basis of the $AUC_{0-\infty}$ values after being intravenously and orally administered, the oral bioavailability of **24** in rat was estimated to be 33.7%. The favorable pharmacokinetic data indicated the possible oral efficacy of these compounds in vivo.

Given the compelling data from both the in vitro efficacy and pharmacokinetics studies, compound **24** was further investigated in an animal model. We chose the insulin-resistant DIO mouse model, which is commonly used as an obese rodent model of type 2 diabetes. Metformin, the front-line drug for the treatment of type 2 diabetes, served as a positive control in the experiments. The DIO mice were administered compound **24** (15, 50, and 150 mg/kg) or metformin (150 mg/kg) by daily oral gavage for 4 weeks. All three dose treatment groups displayed apparent reductions in plasma triglyceride (Table 3). The magnitude of the reduction in plasma triglyceride was similar to the reduction caused by metformin. Glucose tolerance test demonstrated that both 50 and 150 mg/kg of **24** markedly improved glucose tolerance, whereas the lower dose (15 mg/kg) showed a tendency toward improvement. The area under the curve for the 50 and 150 mg/kg of **24** treatment group was attenuated by 30% when compared with the vehicle group; the extent of this effect was similar to that of metformin. Insulin tolerance test revealed that treatment with all three doses of **24** strikingly alleviated insulin resistance in DIO mice when compared to vehicle group. This result suggests that **24** treatment in vivo improved glucose tolerance and alleviated insulin resistance. We next measured the expression levels of genes related to fatty acid oxidation and mitochondrial

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Table 3. Compound 24 Improves the Glucose and Lipid Metabolism of DIO Mice after 4 Weeks of Treatment[a]

compound (mg/kg)	GTT AUC[f]	ITT AUC[g]	plasma TG (mM)
24 (15)	2953.2 ± 374.5[b]	8339.7 ± 268.1[c]	0.52 ± 0.02[d]
24 (50)	2820.0 ± 147.5[d]	8165.6 ± 142.4[c]	0.52 ± 0.02[c]
24 (150)	2861.8 ± 71.4[e]	7533.8 ± 450.9[d]	0.54 ± 0.02[c]
vehicle	3533.8 ± 70.5	9262.5 ± 385.8	0.62 ± 0.02
met[h] (150)	2691.8 ± 312.3[c]	ND[i]	0.50 ± 0.02[d]

[a]The results are presented as the mean ± SE. [b]#, $p < 0.1$. [c]*, $p < 0.05$. [d]**, $p < 0.01$. [e]***, $p < 0.001$ compared with vehicle ($n = 7–10$). [f]GTT, glucose tolerance test; AUC, area under curve; TG, triglyceride. [g]ITT, insulin tolerance test. [h]Met, metformin. [i]ND, not determined.

biogenesis in muscles. Compound **24** treatment significantly upregulated the expression levels of genes related to fatty acid uptake (such as fatty acid binding protein 3 (FABP3)) and fatty acid oxidation (such as carnitine palmitoyltransferase 1 and 2 (CPT1 and CPT2), medium chain acyl CoA dehydrogenase (MCAD), long chain acyl CoA dehydrogenase (LCAD), pyruvate dehydrogenase kinase isozyme 4 (PDK4), and uncoupling protein 2 (UCP2)) (Supporting Information Figure 3). Moreover, genes involved in mitochondrial biogenesis such as peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) and cytochrome c oxidase subunit 5b (COX5b) were also upregulated following **24** treatment. These results suggest that **24** likely improves insulin sensitivity by increasing fatty acid oxidation in muscles.

The goals of this study were to improve the efficacy and potency of hit compound **5**, improve the pharmacokinetics in order to obtain active lead compound in vivo, and better understand the SAR. After initial structural modification of the right side, the left side, and the linking part of compound **5**, a novel series of alkene oxindole derivatives were found to stimulate the recombinant AMPK heterotrimers $\alpha2\beta1\gamma1$, dose-dependently stimulate AMPK and ACC phosphorylation, and increase glucose uptake in L6 myotubes. Because of the improved bioavailability of lead compound **24** and its in vitro biological activity, the beneficial metabolic effects were confirmed in insulin resistance DIO mice. Compound **24** was further tested in preliminary ADME-Tox assays and showed favorable profiles. The activity profiles shown by **24**, at both cellular and animal models, recommend an alkene oxindole series of AMPK activators for further study in the quest for new medications in metabolic diseases, particularly in the treatment of diabetes.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AMPK, adenosine 5′-monophosphate (AMP)-activated protein kinase; ATP, adenosine-5′-triphosphate; ACC, acetyl-CoA carboxylase; LKB1, liver kinase B1; CaMKK, calcium/calmodulin-dependent protein kinase kinase; AICAR, 5-amino-imidazole-4-carboxamide riboside; ZMP, 5-amino-4-imidazole-carboxamide ribotide; SAR, structure−activity relationship; DIO, diet induced obese; CPT1, carnitine palmitoyltransferase 1; CPT2, carnitine palmitoyltransferase 2; MCAD, medium chain acyl CoA dehydrogenase; LCAD, long chain acyl CoA dehydrogenase; PDK4, pyruvate dehydrogenase kinase isozyme 4; UCP2, uncoupling protein 2; FABP3, fatty acid binding protein 3; PGC1α, peroxisome proliferator-activated receptor γ coactivator 1α; CytoC, cytochrome c; COX5b, cytochrome c oxidase subunit 5b

■ REFERENCES

(1) Steinberg, G. R.; Kemp, B. E. AMPK in health and disease. *Physiol. Rev.* **2009**, *89*, 1025−78.

(2) Hardie, D. G. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 774−785.

(3) Witczak, C. A.; Sharoff, C. G.; Goodyear, L. J. AMP-activated protein kinase in skeletal muscle: from structure and localization to its role as a master regulator of cellular metabolism. *Cell. Mol. Life Sci.* **2008**, *65*, 3737−3755.

(4) Hardie, D. G. AMP-activated protein kinase as a drug target. *Annu. Rev. Pharmacol. Toxicol.* **2007**, *47*, 185−210.

(5) Friedman, J. M.; Halaas, J. L. Leptin and the regulation of body weight in mammals. *Nature* **1998**, *395*, 763−770.

(6) Muoio, D. M.; Dohm, G. L.; Fiedorek, F. T., Jr.; Tapscott, E. B.; Coleman, R. A. Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* **1997**, *46*, 1360−1363.

(7) Yamauchi, T.; Kamon, J.; Waki, H.; Terauchi, Y.; Kubota, N.; Hara, K.; Mori, Y.; Ide, T.; Murakami, K.; Tsuboyama-Kasaoka, N.; Ezaki, O.; Akanuma, Y.; Gavrilova, O.; Vinson, C.; Reitman, M. L.; Kagechika, H.; Shudo, K.; Yoda, M.; Nakano, Y.; Tobe, K.; Nagai, R.; Kimura, S.; Tomita, M.; Froguel, P.; Kadowaki, T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat. Med.* **2001**, *7*, 941−946.

(8) Fryer, L. G.; Parbu-Patel, A.; Carling, D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *J. Biol. Chem.* **2002**, *277*, 25226−25332.

(9) Brunmair, B.; Staniek, K.; Gras, F.; Scharf, N.; Althaym, A.; Clara, R.; Roden, M.; Gnaiger, E.; Nohl, H.; Waldhausl, W.; Furnsinn, C. Thiazolidinediones, like metformin, inhibit respiratory complex I: a common mechanism contributing to their antidiabetic actions? *Diabetes* **2004**, *53*, 1052−1059.

(10) Cool, B.; Zinker, B.; Chiou, W.; Kifle, L.; Cao, N.; Perham, M.; Dickinson, R.; Adler, A.; Gagne, G.; Iyengar, R.; Zhao, G.; Marsh, K.; Kym, P.; Jung, P.; Camp, H. S.; Frevert, E. Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. *Cell Metab.* **2006**, *3*, 403−16.

(11) Gomez-Galeno, J. E.; Dang, Q.; Nguyen, T. H.; Boyer, S. H.; Grote, M. P.; Sun, Z. L.; Chen, M. W.; Craigo, W. A.; van Poelje, P. D.; MacKenna, D. A.; Cable, E. E.; Rolzin, P. A.; Finn, P. D.; Chi, B.; Linemeyer, D. L.; Hecker, S. J.; Erion, M. D. A potent and selective

AMPK activator that inhibits de novo lipogenesis. *ACS Med. Chem.Lett.* **2010**, *1*, 478−482.
(12) Guh, J. H.; Chang, W. L.; Yang, J.; Lee, S. L.; Wei, S.; Wang, D.; Kulp, S. K.; Chen, C. S. Development of novel adenosine monophosphate-activated protein kinase activators. *J. Med. Chem.* **2010**, *53*, 2552−2561.
(13) Pang, T.; Xiong, B.; Li, J. Y.; Qiu, B. Y.; Jin, G. Z.; Shen, J. K.; Li, J. Conserved alpha-helix acts as autoinhibitory sequence in AMP-activated protein kinase alpha subunits. *J. Biol. Chem.* **2007**, *282*, 495−506.
(14) Enchev, V.; Markova, N.; Angelova, S. Ab initio study of 2,4-substituted azolidines. II. Amino-imino tautomerism of 2-amino-thiazolidine-4-one and 4-aminothiazolidine-2-one in water solution. *J. Phys. Chem. A* **2005**, *109*, 8904−8913.
(15) Chowdhry, M. M.; Mingos, D. M. P.; White, A. J. P.; Williams, D. J. 15N NMR and crystal structure studies of 5-(2-pyridylmethylene)pseudothiohydantoin; dipolar dephasing experiments for establishing the preferred tautomer in the solid and solution states. *J. Chem. Soc., Perkin Trans. 2* **2000**, 2265−2268.
(16) Kalgutkar, A. S.; Soglia, J. R. Minimising the potential for metabolic activation in drug discovery. *Expert Opin. Drug Metab. Toxicol.* **2005**, *1*, 91−142.
(17) Kalgutkar, A. S.; Gardner, I.; Obach, R. S.; Shaffer, C. L.; Callegari, E.; Henne, K. R.; Mutlib, A. E.; Dalvie, D. K.; Lee, J. S.; Nakai, Y.; O'Donnell, J. P.; Boer, J.; Harriman, S. P. A comprehensive listing of bioactivation pathways of organic functional groups. *Curr. Drug Metab.* **2005**, *6*, 161−225.
(18) Boelsterli, U. A.; Ho, H. K.; Zhou, S.; Leow, K. Y. Bioactivation and hepatotoxicity of nitroaromatic drugs. *Curr. Drug Metab.* **2006**, *7*, 715−727.
(19) Pinto, A.; Neuville, L.; Retailleau, P.; Zhu, J. Synthesis of 3-(diarylmethylenyl)oxindole by a palladium-catalyzed domino carbopalladation/C−H activation/C−C bond-forming process. *Org. Lett.* **2006**, *8*, 4927−30.
(20) Yanada, R.; Obika, S.; Inokuma, T.; Yanada, K.; Yamashita, M.; Ohta, S.; Takemoto, Y. Stereoselective synthesis of 3-alkylideneoxindoles via palladium-catalyzed domino reactions. *J. Org. Chem.* **2005**, *70*, 6972−6975.
(21) Cheung, W. S.; Patch, R. J.; Player, M. R. A tandem Heck-carbocyclization/Suzuki-coupling approach to the stereoselective syntheses of asymmetric 3,3-(diarylmethylene)indolinones. *J. Org. Chem.* **2005**, *70*, 3741−3744.