

## Discovery of Pyridones As Oral AMPK Direct Activators

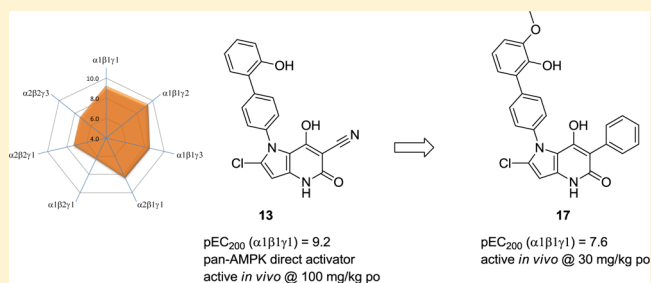
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## Supporting Information

**ABSTRACT:** AMP-activated protein kinase (AMPK) is an evolutionarily conserved fuel-sensing enzyme that is activated in shortage of energy and suppressed in its surfeit. AMPK activation stimulates fatty acid oxidation, enhances insulin sensitivity, alleviates hyperglycemia and hyperlipidemia, and inhibits proinflammatory changes. Thus, AMPK is a well-received therapeutic target for type 2 diabetes and other metabolic disorders. Here, we will report the discovery of pyrrolopyridone derivatives as AMPK direct activators. We will illustrate the synthesis and structure–activity relationships of the series as well as some pharmacokinetic results. Some compounds exhibited encouraging oral exposure and were evaluated in a mouse diabetic model. Compound 17 showed oral activity at 30 mg/kg on blood glucose.

**KEYWORDS:** AMPK direct activator, pyridone, P-gp substrate, type 2 diabetes



AMPK, a well-known heterotrimeric serine/threonine-protein kinase, is a key player in regulating energy metabolism. Because of the different existing subunit isoforms, 12 different AMPK complexes can theoretically exist. AMPK has been established as a sensor and regulator of cellular energy homeostasis.<sup>1,2</sup> Allosteric activation of this kinase due to rising AMP levels occurs in states of cellular energy depletion. The resulting serine/threonine phosphorylation of target enzymes leads to an adaptation of cellular metabolism to the low energy state. The net effect of AMPK activation induced changes is inhibition of ATP consuming processes and activation of ATP generating pathways and therefore regeneration of ATP stores. Examples of AMPK substrates include acetyl-CoA-carboxylase (ACC) and HMG-CoA-reductase.<sup>3</sup> Phosphorylation and therefore inhibition of ACC leads to a decrease in fatty acid synthesis (ATP-consuming) and at the same time to an increase in fatty acid oxidation (ATP-generating). Phosphorylation and resulting inhibition of HMG-CoA reductase leads to a decrease in cholesterol synthesis. Other substrates of AMPK include hormone sensitive lipase,<sup>4</sup> glycerol-3-phosphate acyltransferase,<sup>5</sup> and malonyl-CoA decarboxylase,<sup>6</sup> some of which are potential drug targets for components of metabolic syndrome. Additional processes that are believed to be regulated through AMPK activation, but for which the exact AMPK substrates have not been identified, include stimulation of glucose transport in skeletal muscle and expressional regulation of key genes in fatty acid and glucose metabolism in liver.<sup>1,2,7</sup> For example, decreased expression of glucose-6-phosphatase,<sup>8</sup> a key enzyme in hepatic glucose production, and SREBP-1c,<sup>9</sup> a key

lipogenic transcription factor, has been found following AMPK stimulation.

The activation of AMPK is deemed to be an attractive pharmacological approach for the treatment of type 2 diabetes and other metabolic disorders. More recently, AMPK has also been suggested as a potential target for pharmacological intervention in cancer.<sup>10</sup> A plethora of small molecules have been reported to activate AMPK, but most of them in an indirect manner. Only a few were described to directly activate AMPK by allosteric activation of its catalytic activity but also by protection against AMPK-Thr172 dephosphorylation<sup>11,12</sup> including Metabasis AMP mimetic compound 2<sup>13</sup> and Abbott compound A769662 1.<sup>14</sup> Unfortunately, A769662 1 was found to activate only AMPK β1-containing heterotrimers,<sup>15</sup> and neither compound 2 or A769662 1 are useful as oral drug to explore the effects of AMPK activation utility in long-term treatments.

Our early discovery program led us to investigate the pyridone core starting from A769662, 1. We focused our efforts on replacing the thiophene ring of the Abbott compound 1 by 5-membered ring heterocycles in order to improve oral absorption and selectivity profile over the AMPK heterotrimers.

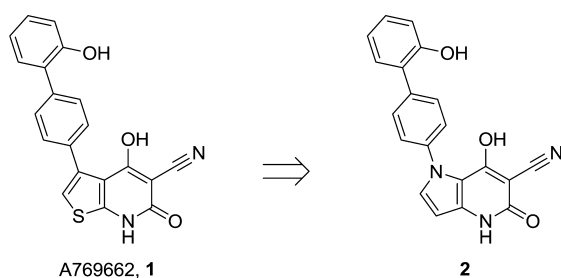
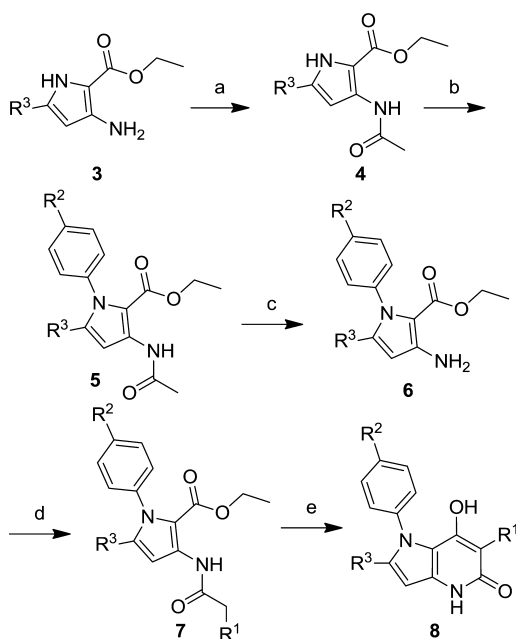
Our initial efforts allowed us to identify the pyrrolopyridone 2 as a new scaffold for AMPK direct activation (Chart 1).<sup>16</sup>

Shown in Scheme 1 is the general synthetic route for preparing the pyrrolopyridones discussed in this letter.<sup>17</sup> 3-

Received: April 24, 2013

Accepted: May 17, 2013

Published: May 17, 2013

**Chart 1. Identification of Pyrrolopyridones As AMPK Direct Activators****Scheme 1. General Synthetic Route for Pyrrolopyridones<sup>a</sup>**

<sup>a</sup>Reagents and conditions: (a) AcCl, Et<sub>3</sub>N, DCM, RT; (b) R<sup>2</sup>PhB(OH)<sub>2</sub>, Cu(OAc)<sub>2</sub>, pyridine or Et<sub>3</sub>N, air, DCM, RT; (c) HCl, EtOH, reflux; (d) R<sup>1</sup>CH<sub>2</sub>COCl, Et<sub>3</sub>N, DCM, RT or R<sup>1</sup>CH<sub>2</sub>CO<sub>2</sub>H, Et<sub>3</sub>N, coupling reagent, acetonitrile, RT; (e) NaH or KHMDS in THF or *t*-BuOK in DMSO.

Amino-pyrroles **3** were first protected as acetates **4** using acetyl chloride in the presence of triethylamine in DCM at RT. A Chan–Lam coupling using standard conditions was then performed to introduce the para-substituted phenyl ring leading to compounds **5** in good yields. Deprotection in acidic conditions of the acetate gave the amino-pyrroles **6** that were then subjected to peptide-coupling conditions to lead to amides **7**. Finally, cyclization of compounds **7** in the presence of strong base afforded the desired pyrrolopyridones **8**. In some cases, the R<sup>2</sup> substituent is further elaborated either from compound **6** or **8** using known chemistries as shown in the Supporting Information section.

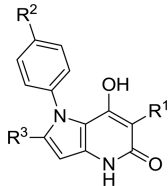
As shown in Table 1, compound **2** is a potent AMPK direct activator of the  $\alpha 1\beta 1\gamma 1$  heterotrimer slightly less potent than A769662, **1**. Compound **2** was directly assessed in mice pharmacokinetic experiment in order to determine the potential of the series. The compound was thus dosed at 30 mg/kg po and gave better blood exposure and oral bioavailability than **1** but displayed a higher blood clearance. Having identified pyrrolopyridones as a new interesting template activating AMPK, the main objective of the medicinal

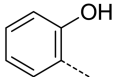
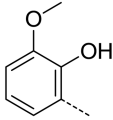
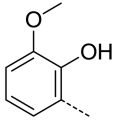
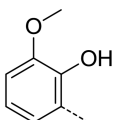
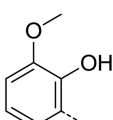
chemistry efforts from **2** was to introduce oral bioavailability by reducing blood clearance and improve absorption for this series of compounds. We first tried to replace the phenol in R<sup>2</sup> with a methoxy group to try to reduce clearance; potency dropped dramatically whatever the position on the phenyl ring is (compounds **9** to **11**). When substituents on compounds **2** and **10** were mixed, compound **12** was found to be potent with a reduced blood clearance of 2.4 mL/min/kg. Probably due to its very low permeability, oral bioavailability of **12** was still very low. Introduction of a chlorine atom at position 2 of the pyrrole ring (compound **13**) led to a nice increase of potency giving us the most potent compound of the series. This extra substituent did not improve permeability nor blood clearance, but oral exposure of **13** started to be of interest for such a potent activator. Replacement of the guaiacol moiety by a tolyl group (compound **14**) gave a less potent compound but with an improved pharmacokinetic profile. Compound **14** exhibited a very low blood clearance and a slightly enhanced permeability that resulted in a very nice oral exposure and an oral bioavailability of 14%. The top phenyl ring could be replaced with a thiophene that gave a potent compound **15**. Again, despite a low permeability, this compound exhibited a nice oral exposure and an oral bioavailability of 21%. All the compounds from this series showed very high plasma protein binding (>99%) that could be attributed in part to the very acidic nature of the 3-cyanopyridone ring (pK<sub>a</sub> for compound **15** was measured lower than 2). We thought of modulating the pK<sub>a</sub> of the ring by replacing the cyano group with a phenyl ring. Compound **16**, having a substituted phenyl moiety attached to the pyrrole ring displayed, reduced potency and had no real improvement in plasma protein binding compared to the previous compounds. Nevertheless, it showed a nice permeability that could come from a higher pK<sub>a</sub> of the pyridone ring, resulting in an oral bioavailability of 50%. When the guaiacol moiety was introduced on this template, compound **17** exhibited an improved potency compared to compound **16**. Acidic pK<sub>a</sub> was measured at 6.0 for this compound proving our hypothesis on the cyano group driving the pK<sub>a</sub> and thus permeability down. Mouse pharmacokinetics was found to be very encouraging for **17** displaying a very low blood clearance and an oral bioavailability of 15%. Finally, introduction of a carboxylic acid on the meta position of the right-hand side phenyl ring (compound **18**) gave a real improvement in potency along with a loss of permeability. This resulted in a very poor pharmacokinetic profile compared to the previous compound.

Despite improvements on most parameters, finding potent AMPK activators with high oral absorption remained challenging. Given the low permeability of this series of compounds, we then assessed their susceptibility against P-glycoprotein (P-gp). P-gp is an ATP-dependent efflux pump with broad substrate specificity that could be the cause of their absorption issue along with their poor permeability.

We selected compounds **13**, **15**, and **17** for further evaluation in mice pharmacokinetics where the compounds (5 mg/kg for **13** and **15**; 30 mg/kg for **17**) were coadministered with P-gp inhibitor GF120918<sup>18</sup> (30 mg/kg). Figure 1 shows a marked improvement of mean blood AUC for the 3 compounds under those conditions suggesting that compounds of this series are P-gp substrate. The low permeability compound **13** shows a 10-fold increase in blood exposure when codosed with GF120918. Compound **15** exhibited the same level of improvement in oral exposure. Compound **17**, probably due to a reduced affinity to

Table 1. SAR Results for Pyrrolopyridones 2–18



Cmpd	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	AMPK hum. rec. $\alpha 1\beta 1\gamma 1^a$ pEC <sub>200</sub>	Permeability <sup>b</sup> nm/s	Solubility <sup>c</sup> $\mu\text{g/mL}$	Mice PPB %	Mouse Pharmacokinetics <sup>d</sup>		
								AUC <sub>po</sub> ng.h/mL	Cl <sub>iv</sub> mL/min/kg	F %
1		A769662		8.5	<3	500	98.7	80	24	2
2	CN		H	7.8	-	-	-	1492 <sup>e</sup>	34	10
9	CN	4-methoxyphenyl	H	6.8	<0.8	59	-	-	-	-
10	CN	3-methoxyphenyl	H	5.3	-	-	-	-	-	-
11	CN	2-methoxyphenyl	H	5.5	-	-	-	-	-	-
12	CN		H	8	<0.4	500	99.3	70	2.4	<1
13	CN		Cl	9.2	<3	425	>99.9	892	3.0	3
14	CN	4-tolyl	Cl	7.5	4.8	129	>99.9	16996	0.7	14
15	CN	3-thienyl	Cl	8.1	<0.7	186	>99.9	19491	0.9	21
16	Ph	Et	Cl	6.3	150	20	99.8	3408	12	50
17	Ph		Cl	7.6	110	25	>99.9	41346	0.3	15
18			Cl	8.7	<0.6	325	>99.9	403	1.0	0.5

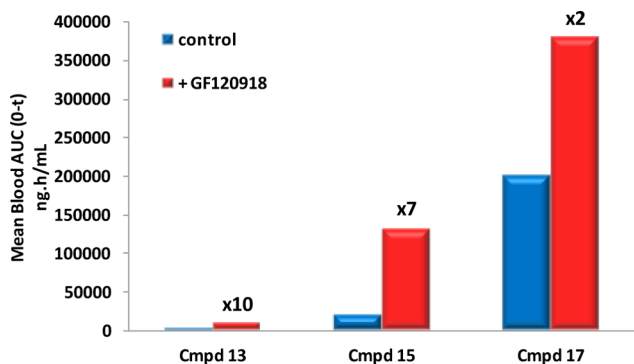
<sup>a</sup>Hum. rec. AMPK  $\alpha 1\beta 1\gamma 1$  (Invitrogen) was used in a FRET assay format (ZLyte, Invitrogen). pEC<sub>200</sub> =  $-\log(\text{compound concentration leading to a 2-fold AMPK activity increase})$ . All data are means of at least 2 independent experiments. <sup>b</sup>Permeability: Pampa (Parallel Artificial Membrane Permeability Assay). <sup>c</sup>Solubility: soluble fraction of oral formulation (see below) following its manufacture by ball milling from solid powder (sol max. 500 mg/mL). <sup>d</sup>Mouse PK: po @ 5 mg/kg in suspension in HPMC K100 0.5%/Tween 80 0.1% in buffer pH 7 and iv @ 1 mg/kg in solution in DMSO/hydroxypropyl- $\beta$ -cyclodextrin 20% in buffer pH 7 (5:95). po @ 30 mg/kg in suspension in HPMC K100 0.5%/Tween 80 0.1% in buffer pH 7. <sup>e</sup>po @ 30 mg/kg in suspension in HPMC K100 0.5%/Tween 80 0.1% in buffer pH 7.

P-gp, shows only a 2-fold increase in blood AUC (see Supporting Information Table S1 for detailed data).

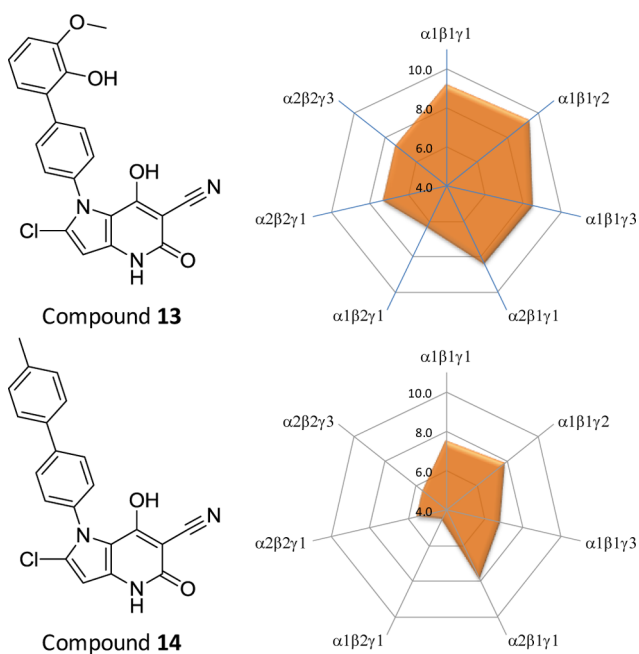
Having identified compounds from the pyridone series that were compatible with an oral route, we turned our efforts on their AMPK heterotrimers selectivity. As explained earlier in this letter, in order to explore the AMPK pathway in vivo, a compound activating all or most AMPK heterotrimers seems attractive. Compound A769662, **1**, has been described as  $\beta 1$ -selective AMPK heterotrimers,<sup>15</sup> and we assessed compounds from the pyrrolopyridone series on the seven AMPK heterotrimers that were available to us (Figure 2). Compounds **13**, the most potent compound of the series on the  $\alpha 1\beta 1\gamma 1$  AMPK heterotrimer, proved to activate all seven AMPK

heterotrimers we tested. Despite the fact that compound **13** is more potent on the  $\beta 1$ -containing heterotrimers, it is activating  $\alpha 2\beta 2\gamma 1$  and  $\alpha 2\beta 2\gamma 3$  with a pEC<sub>200</sub> = 7.3 for both heterotrimers (see Supporting Information for complete heterotrimers data). Even compound **14**, a weaker compound on the  $\alpha 1\beta 1\gamma 1$  AMPK heterotrimer, was able to modestly activate some  $\beta 2$ -containing heterotrimers ( $\alpha 2\beta 2\gamma 1$  and  $\alpha 2\beta 2\gamma 3$  with a pEC<sub>200</sub> = 5.5, but for  $\alpha 1\beta 2\gamma 1$ , pEC<sub>200</sub> < 4.5). Compounds **15** and **17** were also tested on  $\beta 2$ -containing heterotrimer  $\alpha 2\beta 2\gamma 1$  and found active (see Supporting Information).

Compounds **13**, **15**, and **17** were selected for further in vivo profiling given their additional PK profiles. Compounds **13** and **15** were evaluated in a chronic oral treatment in ob/ob mice for

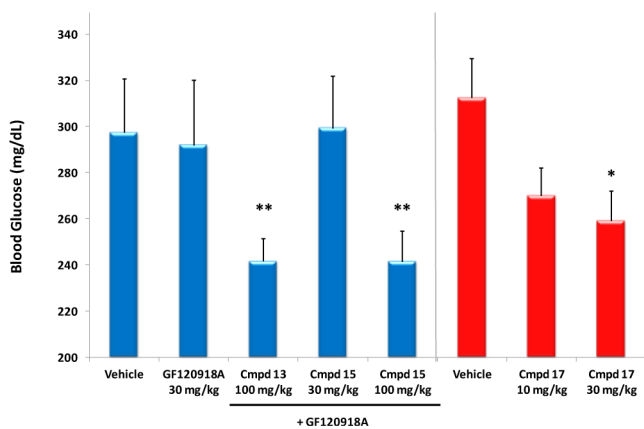


**Figure 1.** Influence of P-gp inhibition on mean blood AUC of pyrrolopyridones 13, 15, and 17.



**Figure 2.** AMPK heterotrimers profiling for pyridones 13 and 14.

5 days at 30 and 100 mg/kg bid in combination with GF120918. P-gp inhibitor GF120918 by itself has no effect on blood glucose (Figure 3). Both compounds 13 and 15 were able to reduce blood glucose of 55 mg/dL (18% and 17%



**Figure 3.** Compounds 13, 15, and 17 orally reduce blood glucose in ob/ob mice.

reduction of blood glucose, respectively) at the dose of 100 mg/kg ( $P < 0.001$ ) when administered with the P-gp inhibitor highlighting the potential of our AMPK activators for blood glucose control. Compound 17 having a better oral exposure was dosed orally for 5 days bid at 10 and 30 mg/kg without P-gp inhibitor. Blood glucose was monitored 12 h after the last gavage, and compound 17 was found to decrease it significantly by 53 mg/dL (17% reduction of blood glucose) at the dose of 30 mg/kg ( $P < 0.05$ , Figure 3). We only observed a trend for blood glucose reduction (13%) at 10 mg/kg that was not statistically significant.

The objectives of this study were to improve the pharmacokinetics of pyridone compounds in order to obtain active AMPK direct activators in vivo and explore the effects of AMPK activation utility in longer term treatments. After structural modification of the thiophene ring, a novel series of pyrrolopyridones was found to activate directly AMPK heterotrimers with a pan profile. In regard to its adequate oral PK profile, compound 17 was further tested in the animal model of diabetes and showed activity at 30 mg/kg on blood glucose. All of the data suggest that we have identified a series of compounds useful to explore AMPK direct activation in vivo.

## ■ ASSOCIATED CONTENT

### Supporting Information

All experimental procedures and compounds characterization, material and methods about DMPK, in vivo experiment protocol, and AMPK heterotrimers profiling. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Author Contributions

All authors have given approval to the final version of the manuscript.

### Notes

The authors declare the following competing financial interest(s): All authors were GlaxoSmithKline full-time employee at the time when this study was performed.

All studies involving the use of animals were conducted after review by the GlaxoSmithKline (GSK) Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

## ■ ACKNOWLEDGMENTS

Yann Lamotte, Delphine Delannée, Alain Laroze, Anne Bouillot, Van-Loc Nguyen, Nicolas Faucher, Olivier Pineau, Alain Daugan, and Nérina Dodic are warmly acknowledged for their technical assistance and helpful discussions during the course of this study.

## ■ ABBREVIATIONS

AMPK: adenosine 5'-monophosphate-activated protein kinase; ATP: adenosine-5'-triphosphate; ACC: acetyl-coenzyme A carboxylase; HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme

A; SREBP-1c: sterol regulatory element-binding proteins-1c; P-gp: P-glycoprotein; AUC: area under the curve; FRET: fluorescence resonance energy transfer; Pampa: parallel artificial membrane permeability; PK: pharmacokinetic; HPMC: hydroxypropyl methylcellulose

## REFERENCES

- (1) Hardie, D. G.; Hawley, S. A. AMP-activated protein kinase: the energy charge hypothesis revisited. *Bioessays* **2001**, *23*, 1112–1119.
- (2) Kemp, B. E.; Stapleton, D.; Campbell, D. J.; Chen, Z. P.; Murthy, S.; Walter, M.; Gupta, A.; Adams, J. J.; Katsis, F.; van Denderen, B.; Jennings, I. G.; Iseli, T.; Michell, B. J.; Witters, L. A. AMP-activated protein kinase, super metabolic regulator. *Biochem. Soc. Trans.* **2003**, *31*, 162–168.
- (3) Carling, D.; Zammit, V. A.; Hardie, D. G. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. *FEBS Lett.* **1987**, *223*, 217–222.
- (4) Garton, A. J.; Campbell, D. G.; Carling, D.; Hardie, D. G.; Colbran, R. J.; Yeaman, S. J. Phosphorylation of bovine hormone-sensitive lipase by the AMP-activated protein kinase. A possible antilipolytic mechanism. *Eur. J. Biochem.* **1989**, *179*, 249–254.
- (5) Muoio, D. M.; Seefeld, K.; Witters, L. A.; Coleman, R. A. AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-phosphate acyltransferase is a novel target. *Biochem. J.* **1999**, *338*, 783–791.
- (6) Saha, A. K.; Schwarsin, A. J.; Roudit, R.; Masse, F.; Kaushik, V.; Tornheim, K.; Prentki, M.; Ruderman, N. B. Activation of malonyl-CoA decarboxylase in rat skeletal muscle by contraction and the AMP-activated protein kinase activator 5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside. *J. Biol. Chem.* **2000**, *275*, 24279–24783.
- (7) Musi, N.; Goodyear, L. J. Targeting the AMP-activated protein kinase for the treatment of Type 2 diabetes. *Curr. Drug Targets: Immune, Endocr. Metab. Disord.* **2002**, *2*, 119–127.
- (8) Lochhead, P. A.; Salt, I. P.; Walker, K. S.; Hardie, D. G.; Sutherland, C. 5-Aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. *Diabetes* **2000**, *49*, 896–903.
- (9) Zhou, G.; Myers, R.; Li, Y.; Chen, Y.; Shen, X.; Fenyk-Melody, J.; Wu, M.; Ventre, J.; Doebber, T.; Fujii, N.; Musi, N.; Hirshman, M. F.; Goodyear, L. J.; Moller, D. E. Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest.* **2001**, *108*, 1167–1174.
- (10) Kuhajda, F. P. AMP-activated protein kinase and human cancer: cancer metabolism revisited. *Int. J. Obesity* **2008**, *32*, S36–S41.
- (11) Sanders, M. J.; Grondin, P. O.; Hegarty, B. D.; Snowden, M. A.; Carling, D. *Biochem. J.* **2007**, *403*, 139–148 and references cited therein.
- (12) Zhou, G.; Sebat, I. K.; Zhang, B. B. AMPK activators: potential therapeutics for metabolic and other diseases. *Acta Physiol.* **2009**, *196*, 175–190.
- (13) Gomez-Galeno, J. E.; Dang, Q.; Nguyen, T. H.; Boyer, S. H.; Grote, M. P.; Sun, Z.; Chen, M.; 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.
- (14) Cool, B.; Zinker, B.; Chiou, W.; Kifle, L.; Cao, N.; Perham, M.; Dickinson, R.; Adler, A.; Gagne, G.; Lyengar, R.; Zhao, G.; Marsh, K.; Kym, P.; Jung, P.; Camp, H. S.; Frevert, E. *Cell Metab.* **2006**, *3*, 403–416.
- (15) Scott, J. W.; van Denderen, B. J.; Jorgensen, S. B.; Honeyman, J. E.; Steinberg, G. R.; Oakhill, J. S.; Iseli, T. J.; Koay, A.; Gooley, P. R.; Stapleton, D.; Kemp, B. E. Thienopyridone drugs are selective activators of AMP-activated protein kinase  $\beta$ 1-containing complexes. *Chem. Biol.* **2008**, *15*, 1220–1230.
- (16) Mirguet, O.; Bouillot, A.; Daugan, A.; Faucher, N.; Dodic, N.; Sautet, S.; Delannee, D.; Toum, J.; Laroze, A.; Donche, F.; Clement, C.; Nguyen, V.-L.; Pineau, O.; Marques, C.; Lamotte, Y.; Martin, F.; Ajakane, M.; Beaufile, B.; Dudit, Y.; Huet, P.; Trottet, L.; Grondin, P.; Saintillan, Y.; Nicodeme, E. Discovery of oral AMPK direct activators for the treatment of diabetes. Abstracts of Papers, *243rd ACS National Meeting & Exposition*, San Diego, CA, United States, 25–29 March 2012; MEDI-189.
- (17) Mirguet, O. Pyrrolopyridone derivatives as activators of AMPK and their preparation and use in the treatment of diseases. US patent WO2011029855, 2011.
- (18) Hyafil, F.; Vergely, C.; Du Vignaud, P.; Grand-Perret, T. In vitro and in vivo reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative. *Cancer Res.* **1993**, *53*, 4595–4602.