Natural Product Sciences
19(1) : 1-7 (2013)

AMPK Activators from Natural Products: A Patent Review

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Abstract – AMP-activated protein kinase (AMPK) is a major cellular energy sensor and master regulator of metabolic homeostasis. On activation, this cellular fuel sensing enzyme induces a series of metabolic changes to balance energy consumption via multiple downstream signaling pathways controlling nutrient uptake and energy metabolism. This pivotal role of AMPK has led to the development of numerous AMPK activators which might be used as novel drug candidates in the treatment of AMPK related disorders, diabetes, obesity, and other metabolic diseases. Consequently, a number of patents have been published on AMPK activators from natural products and other sources. This review covers the patented AMPK activators from natural products and their therapeutic potential in treatment or prevention of metabolic diseases including diabetes and obesity.
Keywords – AMPK activators, Metabolic syndrome, Patents, Natural products

Introduction

AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that acts as an intracellular energy sensor. Over the last few years, accumulating evidence has demonstrated that AMPK is a major regulator of cellular and whole body energy homeostasis that coordinates metabolic pathways in order to balance nutrient supply with energy demand. However, it is activated by an increase in the cellular AMP: ATP ratio induced by metabolic stress, hormone and nutrient signals (Corton *et al.*, 1994). Once activated AMPK phosphorylates a number of downstream substrates, the overall effect of which is to switch on catabolic pathways that generate ATP and switch off ATP consuming anabolic pathways by acute regulation of the activity of key enzymes in metabolism and chronic regulation of the expression of key transcription factors (Hardie, 2007; Woods *et al.*, 2000). This pivotal role of AMPK places it in an ideal position of therapeutic drug target in the treatment of diabetes, obesity and other metabolic disorders. Therefore, the intense search for novel AMPK activators by rational drug design, screening of vast chemical libraries and testing of various plant extracts has produced numerous promising compounds; and a number of patents have been published on AMPK activators. Since, there is a renewed interest on AMPK activators from natural products; therefore, in this review we will discuss recently published patented AMPK activators from natural products and their therapeutic potential in metabolic disorders including diabetes and obesity.

Structure of AMPK – AMPK is a highly evolutionarily conserved serine/threonine kinase and found in all eukaryotic species. It exists as a heterotrimeric complex consisting of a catalytic (α1 or α2) subunit and two regulating (β1 or β2 and γ1, γ2 or γ3) subunits, all of which are encoded by separate genes, therefore, at least 12 combinations are possible (Hardie, 2003). At the molecular level, (Fig. 1) the catalytic α subunits contain a classical serine/threonine kinase domain at the N-terminus while a regulatory domain at the C-terminus, which is involved in complex formation with β and γ subunits (Hardie *et al.*, 2006). The β subunits contain C-terminal region that interacts with α and γ subunits and serves as the scaffold of the heterotrimeric complex (Carling, 2004). In addition, the central part of β subunits contain a specific sequence 'N-isomylase domain' required for AMPK complexes binding to glycogen (Hudson *et al.*,

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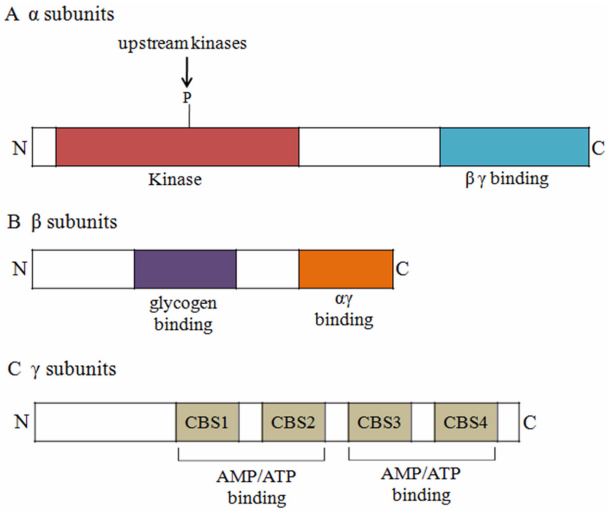


Fig. 1. Typical domain structure of the α, β, and γ subunits of AMPK.

2003; Polekhina *et al.*, 2003). The γ subunits contain N-terminal four tandem repeats of cystathionine-β-synthase sequences (CBS) to form two Bateman domains, which selectively bind adenosine containing molecules, such as AMP or ATP (Bateman, 1997; Scott *et al.*, 2004).

AMPK in metabolic functions – AMPK is ubiquitously expressed and plays an important role in the peripheral metabolism of the skeletal muscle, liver, fat, myocardium and other tissues. It is activated in response to ATP depletion, which causes a concomitant increase in the AMP: ATP ratio (Corton *et al.*, 1994). On activation, AMPK phosphorylates several down-stream substrates, the overall effect of which is, to restore the AMP/ATP ratio by switching on ATP-generating pathways (e.g. fatty acid oxidation and glycolysis) as well as switching off ATP-consuming pathways (e.g. fatty acid synthesis and cholesterol synthesis) that are not essential for short term cell survival (Hardie, 2007; Kahn *et al.*, 2005). Therefore, AMPK plays a key role in regulation of metabolic functions such as glucose and lipid metabolism.

Glucose homeostasis – Glucose homeostasis is maintained by a balance between hepatic glucose production and glucose uptake by peripheral tissues. AMPK exerts a potent effect on glucose metabolism. On activation via muscle contraction, AMPK enhances glucose uptake through the translocation of glucose transporter 4 (GLUT4) to the cell membrane and regulation of GLUT4 gene expression (Holmes *et al.*, 1999). Interestingly, this effect depends on the muscle fiber type. For instance, AMPK increases glucose uptake and also hexokinase II expression in fast-twitching (glycolytic) muscles whereas these effects are absent in slow-twitching (oxidative) soleus muscle (Derave *et al.*, 2000; Holmes *et al.*, 1999; Wright *et al.*, 2005). Furthermore, in muscles, AMPK plays an important role in mitochondrial biogenesis upon activation of nuclear respiratory factor 1 (NRF-1) and peroxisome proliferator activated receptor γ (PPARγ) co-activator 1 (PGC-1) (Zong *et al.*, 2002). Similar effects can be observed in skeletal muscle after activation of AMPK with 5-amino-4-imidazole carboxamide riboside (AICAR) (Winder *et al.*, 2000).

AMPK, in liver, controls glucose homeostasis through the inhibition of gluconeogenesis. It is achieved via the phosphorylation of transcriptional coactivator transducer of regulated cAMP response element-binding (CREB) protein activity 2 (TORC2) and inhibitory gene expression for key gluconeogenic enzymes, glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), and for the transcriptional co-activator peroxisome proliferator activated receptor γ co-activator 1-alpha (PGC-1α) (Cool *et al.*, 2006; Koo *et al.*, 2005; Lochhead *et al.*, 2000).

Lipid metabolism – AMPK is a key modulator of lipid metabolism. In fact, fatty acid synthesis pathway, in the liver and hypothalamus is one of the best characterized targets for AMPK. Upon activation, AMPK phosphorylates and inhibits ACC specifically ACC1, decreases fatty acid synthase (FAS) expression and activates malonyl-CoA carboxylase, thereby leading to a decrease in fatty acid synthesis (Kahn *et al.*, 2005; López *et al.*, 2007; Woods *et al.*, 2000). In liver and hypothalamus as well as in muscle, activated AMPK stimulates fatty acid oxidation by decreasing malonyl-CoA levels through the inhibition of ACC2 (Kahn *et al.*, 2005; López *et al.*, 2007; Merrill *et al.*, 1997). This leads to an increase in carnitine palmitoyl transferase 1 (CPT1) activity and the subsequent activation of fatty acid oxidation (Kahn *et al.*, 2005; López *et al.*, 2007). AMPK is also known to inhibit the activity of HMG-CoA reductase and glycerol phosphate acyl transferase via phosphorylation, which are involved in synthesis of cholesterol and triacylglycerol (Henin *et al.*, 1995; Muoio *et al.*, 1999). Moreover, AMPK also inhibits lipolysis through direct phosphorylation of hormone-sensitive lipase (HSL) and by the blocking of protein kinase A (PKA)-induced HSL activation (Corton *et al.*, 1995; Dagon *et al.*, 2006; Sullivan *et al.*, 1994).

Targeting AMPK activators as treatments for metabolic disorders – The AMPK pathway and modulation of energy balance through the AMPK system presents an attractive therapeutic target for intervention in many conditions of disordered energy balance such as diabetes, obesity and other metabolic disorders. The

metabolic syndrome is a cluster of metabolic disorders including insulin resistance, hypertriglyceridemia, abdominal obesity, hypertension, reduced levels of the beneficial high density lipoprotein and disturbances in glucose metabolism (Trevisan *et al.*, 1998). Downstream targets of AMPK such as genes regulating carbohydrate metabolism (e.g. glycogen synthase, ChREBP) or lipid metabolism (e.g. HMG-CoA, FAS, ACC) play an important role in features of the metabolic syndrome and therefore AMPK emerged as a potential therapeutic target for treatment.

Some drugs which activate AMPK in peripheral tissues, are approved for treating type 2 diabetes and other aspects of metabolic syndrome (Smiley and Umpierrez, 2007). Perhaps, the best known are the biguanide metformin, the thiazolidinedione (TZD) rosiglitazone and metformin-rosiglitazone combination therapy. Metformin inhibits hepatic glucose production, whereas rosiglitazone increases insulin-dependent glucose uptake in skeletal muscle (Hutchinson *et al.*, 2008). Furthermore, these therapies have additional benefits, including decrease in plasma free fatty acids and low-density lipoproteins (LDL) and increase in high-density lipoproteins (HDL) (Smiley and Umpierrez, 2007). Beside these, administration of AICAR in vivo reverses many of the defects associated with the metabolic syndrome in animal models. It's effects include improvements in glucose tolerance, reduced plasma triglyceride and free fatty acid levels, increased whole-body glucose disposal, decreased hepatic glucose output and even a tendency towards a reduction in abdominal fat (Bergeron *et al.*, 2001; Buhl *et al.*, 2001; Song *et al.*, 2002). However, AMPK has role in others aspects of metabolic syndrome. Resveratrol, a naturally occurring polyphenol, has been shown to afford protection to ischemic cardiac cells by inhibiting the build-up of reactive oxygen species. More recently, it has been demonstrated in some cell types that this protection is made possible through the activation of AMPK (Hwang *et al.*, 2008). Finally it is interesting to note that, treatment of obese mice with a new AMPK activator A-769662, reduces hepatic expression of PEPCK, G6Pase and FAS, lowers plasma glucose, reduces body weight gain and decreases both plasma and liver triglyceride levels (Cool *et al*., 2006), suggesting that this drug or a similar one could be an alternative approach for the treatment of type 2 diabetes and other metabolic disorders.

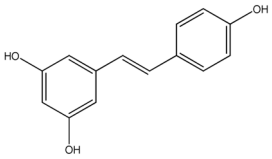
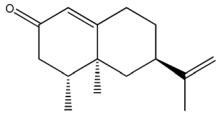
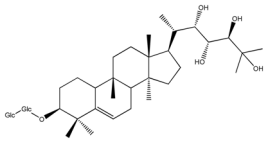
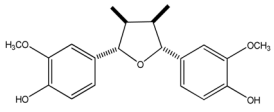
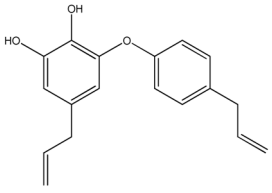
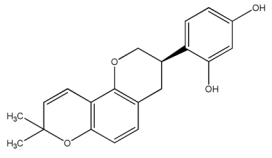
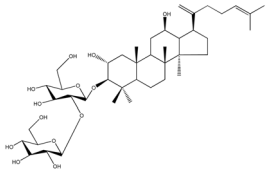
Patents describing AMPK activators from natural products – Recently a number of patents (summarized in Table 1) have been published on natural AMPK activators which have potential in metabolic disorders including diabetes and obesity.

Resveratrol – Resveratrol (trans-3,5,4'-trihydroxystilbene) is a dietary polyphenol compound, found in a wide variety of plant species including grapes, red wines, berries, and peanuts. Recently, resveratrol has been reported as a potent AMPK activator (Ajmo *et al.*, 2008) and useful in the treatment of diabetes and metabolic syndrome (Szkudelska and Szkudelski, 2010). Murase *et al.* in US20110319497 (Murase, 2011b) reported resveratrol as an exercise substitutive agent, which, through AMPK activation, induces energy metabolism and is effective for preventing or ameliorating obesity, diabetes, hyperglycemia, insulin resistance, hypercholesterolemia, hepatic hypertrophy or fatty liver. In this invention, the AMPK activation potential of resveratrol was evaluated on the basis of phosphorylation of AMPK α and β in mouse hepatocyte cell line (Hepa 1-6) and mouse muscle cell line (C2C12) and the result showed that resveratrol activated AMPK α (538%) and AMPK β (168%) at 150 μM, as compared with control in Hepa 1-6 cells while in C2C12 cells, the activation value was 848% (AMPK α) and 425% (AMPK β).

Nootkatone – Nootkatone, [4, 4a, 5, 6, 7, 8-hexahydro-6-isopropenyl-4, 4a-dimethyl-2(3H) naphthalenone], present in grapefruit peels, has received much attention as flavor because of its characteristic flavor and taste of grapefruit. Although, it's physiological activity has hardly been reported, recently, Murase *et al.* found nootkatone as potent AMPK activator (Murase *et al.*, 2010). Furthermore, in US20110118359 (Murase, 2011a) it has been claimed that nootkatone has strong AMPK activating action in muscle cells which promotes glucose and lipid metabolism. In vitro study revealed that nootkatone activated AMPK α (1077%) and AMPK β (358%) at 150 μM, as compared with control in C2C12 muscle cells. In vivo, administration of nootkatone exhibited beneficial effects including reduction of body weight, triglyceride, cholesterol, body fat accumulation, and endurance enhancing and anti-fatigue effects.

Cucurbitane triterpenoid – Ye *et al.* in EP2255816 (Ye *et al.*, 2010) disclosed cucurbitane type triterpenoids, isolated from *Momordica charanita* L (Cucurbitaceae family) and claimed that these compounds may act as glucose uptake stimulator, agonist for translocation of GLUT4 to the cell membrane and AMPK activator. Furthermore, in this invention, four compounds were tested and it was shown that, momordicoside A was capable to increase glucose uptake in L6 muscle cells. Two other compounds, trihydroxycucurbita-5,23(E)-dien-19-al and 22(S),23(R),24(R),25-tetrahydroxycucurbita-5-ene were capable of promoting the translocation of

Table 1. Patented AMPK activators from natural products

Patent no.	Compound	Plant source	Therapeutic Claims	Ref.
US20110319497 (A1)	 Resveratrol	*Vitis amurensis*	Resveratrol is used as antidiabetic and antiobesity agent	(Murase, 2011b)
US20110118359 (A1)	 Nootkatone	*Citrus paradisi*	Nootkatone is used as antidiabetic, antiobesity as well as exercise substituting agent	(Murase, 2011a)
EP2255816 (A1)	 Momordicoside A	*Momordica charanita*	Agent for prevention and treatment of diabetes and obesity	(Ye *et al*., 2010)
US20120083525 (A1)	 Nectandrin B	*Myristica fragrans*	Nectandrin B might be used for prevention and treatment of metabolic syndrome such as obesity, diabetes, and hyperlipidemia	(Oh *et al*., 2012)
US20100125103 (A1)	 Obovatol	*Magnolia obovata*	Obovatol possess high blood sugar lowering effect and therefore it is used as antidiabetic agent	(Huh *et al*., 2010)
WO2007058480 (A1)	 Glabridin	*Glycyrrhiza glabra*	Glabridin is used for diseases which arise from excessive accumulation of surplus calories in the body such as diabetes and obesity	(Park and Yoo, 2007)
US20110015142 (A1)	 Damulin B	*Gynostemma pentaphyllum*	Damulin B possess high antidiabetc and antiobesity effects	(Huh *et al*., 2011)

GLUT4 to the cell membrane and hence increase glucose uptake in cells. In addition, these two compounds showed significant effect in activation of AMPK signaling pathway.

2,5-bis-aryl-3,4-dimethyltetrahydrofuran lignans – Oh *et al*. in US20120083525 (Oh *et al*., 2012) reported 2,5-bis-aryl-3,4-dimethyltetrahydrofuran lignan compounds, isolated from *Myristica fragrans*, as potent AMPK activator which might be useful in the prevention or treatment of AMPK related disorders including diabetes and metabolic syndrome. The isolated compounds were

evaluated for AMPK activation potential and it was shown that, all compounds activated AMPK and ACC significantly. Moreover, in vivo study using high fat diet induced obese mice revealed that; nectandrin B decreased body weight (8.89 g) as compared with control (13.4 g) via AMPK activation.

Obovatol – Obovatol, a phenolic constituent from *Magnolia obovata* is well known for anti-depressive and anti-anxiety effect (Ma *et al.*, 2009). Recently, in US20100125103 (Huh *et al.*, 2010) obovatol has been claimed as potent AMPK activating agents and it might be used in treatment of diabetes and metabolic syndrome. In this invention, in vitro study showed that obovatol and its derivatives activated APMK in L6 myotube cell in concentration dependent manner. Futhermore, oral administration of obovatol to db/db mice for 4 weeks resulted in decreased blood glucose level (the reduced extent: 55.2% compared to the control group (288 ± 27.6, 522 ± 78)).

Glabridin – Glabridin is a dietary isoflavan compound, one of the major active flavonoids in licorice (*Glycyrrhiza glabra*). It has been reported as a novel AMPK activator that would exert therapeutic effects in obesity-related metabolic disorders (Lee *et al.*, 2012). Moreover, Park *et al.* in WO2007058480 (Park and Yoo, 2007), disclosed glabridin as a strong AMPK activator and therefore, potential drug target for diabetes and metabolic syndrome. In vitro study showed that in myoblast cell line C2C12, at 30 μM concentration, glabridin activated AMPK by two fold increase comparing with control DMSO. Furthermore, administration of glabridin resulted in a significant decrease of body weight in C57BL/6JL Lep^{ob}/ Lep^{ob} mice and led to effective control of the blood glucose level in ZDF rats, via AMPK activation.

Damulin A and B – Huh *et al*. in US20110015142 (Huh *et al.*, 2011) reported two novel dammarane type glycosides, named damulin A and B from *Gynostemma pentaphyllum* as strong AMPK activator. In vitro study revealed that both compounds, damulin A and B strongly activate AMPK in cultured L6 myotube cells in concentration dependent manner. Moreover, upon AMPK activation both compound increased β-oxidation (damulin A: 1.8 times; damulin B: 2.6 times) and glucose uptake (damulin A: 42%, damulin B: 79%) with increasing GluT4 translocation to the plasma membrane in L6 myotube cells. Taken together, the results clearly indicated that damulin A and B possess high antidiabetic and antiobesity effect.

Conclusion

As a sensor of cellular energy levels that acts on a diverse array of biological pathways, it is not surprising that AMPK plays a key role in the regulation of cell growth and metabolism. Thus, significant progress has been made in the discovery of novel and promising activators of AMPK and in resolving the mechanism of action of agents that activate AMPK in cells. However, considering the complexity of AMPK biology and the biochemical features associated with AMPK complexes, it is highly challenging to discover complex and/or tissue-selective AMPK activators. Nevertheless, number of recent advances in the field has begun to make AMPK a feasible drug target. Consequently, several natural compounds, reported in last few years, showed promise as AMPK activator which might have exciting potential for use in the treatment of metabolic diseases.

Acknowledgement

This study was supported by Korea Institute for Planning and Evaluation for Technology in Food (iPET), Korea Ministry for Food, Agriculture, Forestry and Fisheries.

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Received March 8, 2013
Revised March 11, 2013
Accepted March 13, 2013