CHAPTER TEN

AMPK Activation in Health and Disease

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1. INTRODUCTION

The maintenance of cellular energy levels is a fundamental biological process. The primary mechanism for detecting and responding to changes in energy state is through the 5′ adenosine monophosphate-activated protein kinase (AMPK) pathway. The enzyme is activated in response to exercise and following treatment with metformin, the first-line therapy for type 2 diabetes mellitus (T2DM). As might be expected from its role, AMPK effects are pleiotropic, impacting multiple metabolic pathways in order to spare and/or generate 5′ adenosine triphosphate (ATP).

The goal of this review is to provide a broad overview of reported AMPK activators and to update the reader on recent advances in the elucidation of AMPK function. Given the breadth of cellular processes impacted by AMPK, the authors have chosen to focus on impacts to metabolic function and potential indications around the treatment of metabolic syndrome and T2DM.

Annual Reports in Medicinal Chemistry, Volume 47
ISSN 0065-7743
http://dx.doi.org/10.1016/B978-0-12-396492-2.00010-2

2. AMPK—ENZYME STRUCTURE AND FUNCTION

AMPK is a Ser/Thr protein kinase consisting of one α-catalytic (2 isoforms), one β-"scaffold" (2 isoforms), and one γ-regulatory (3 isoforms) subunit.[1] Excluding splice variants, 12 distinct AMPK complexes exist. The detailed molecular structure of AMPK has been the subject of considerable investigation. While the field awaits higher resolution structures, available crystallographic studies provide considerable detail on the overall structure and regulation of the complex.[2,3]

AMPK is a "stress" kinase that spares/replenishes depleted cellular energy reserves. Its activity is principally controlled by α-subunit Thr172 phosphorylation. Four Ser/Thr kinases that phosphorylate AMPK (AMPKK) have been identified in mammalian cells: liver kinase B1 (LKB1), calcium/calmodulin-dependent protein kinase kinase β (CAMKKβ), ataxia telangiectasia mutated, and transforming growth factor β-activated kinase 1.[4]

AMPK was so-named based on the ability of 5′ adenosine monophosphate (AMP) to stimulate its activity. Recent studies have established that AMPK is more broadly an "adenylate charge regulated" kinase.[2,3,5] AMP binding to the γ-subunit activates AMPK in three distinct ways: (1) increasing the accessibility of Thr172 for phosphorylation (generating pThr172); (2) inducing a conformation that decreases pThr172 dephosphorylation, and (3) allosterically activating phosphorylated AMPK (pAMPK) up to 2.5-fold. 5′ Adenosine diphosphate (ADP) binding has similar effects, but it cannot intrinsically activate pAMPK. By contrast, Mg^{2+}-ATP binding leads to inactivation by increasing the accessibility of pThr172 to protein phosphatases and by competing for AMP and ADP binding.

AMPK activation triggers phosphorylation of downstream targets, leading to activation or inhibition of target function. Functional consequences can either be immediate or delayed through modulation of transcription factors.[6] On balance, the overriding effect of AMPK action is to inhibit ATP-requiring processes and activate ATP-producing, catabolic processes.

3. MAJOR AMPK-MEDIATED EFFECTS ON LIPID AND CARBOHYDRATE METABOLISM

AMPK activation has profound effects on lipid metabolism.[1,7–9] The enzyme is responsible for phosphorylation and inhibition of both acetyl CoA carboxylase (ACC) and HMG-CoA reductase (the target of the statin class of

hypercholesterolemia drugs).[10] ACC isoforms 1 and 2 are key enzymes regulating lipid metabolism. AMPK-mediated phosphorylation of ACC inhibits fatty acid synthesis and elongation and increases fat oxidation.[11] AMPK also phosphorylates several transcription factors, reducing lipogenic gene expression, for example, sterol regulatory element-binding protein-1c and -2 and carbohydrate responsive element-binding protein.[12,13] Additional data demonstrate that AMPK modulates the synthesis and mobilization of triglycerides (TGs) and increases fatty acid transport into cells.[1,9,14,15] In summary, the net effect of AMPK activation is to decrease lipid synthesis and storage and increase lipid utilization for energy production.

AMPK activation also has major effects on carbohydrate metabolism. Among the most important of these is the AMPK-induced increase in skeletal muscle glucose uptake, which is observed following its activation by exercise and 5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide (AICAR).[16–18] AMPK activation increases glucose uptake in part by increasing the plasma membrane content of glucose transporter 4 (Glut4).[19] This is triggered by phosphorylation of key proteins including tre-2/USP6, BUB2, cdc16 domain family member 1 (TBC1D1), and Akt substrate of 160 kDa (AS160), leading to displacement of Glut4 from its intracellular docking sites.[20] Insulin-stimulated Glut4 translocation is a critical component of the mechanism by which insulin acts to reduce postprandial plasma hyperglycemia.[19] In this manner, AMPK activation can be viewed as an insulin mimic, which has important therapeutic consequences.[21–24] The resulting increased tissue glucose is either stored as glycogen or utilized for glycolysis; both of these processes are stimulated by AMPK activation.[25,26]

During fasting, increased hepatic glucose production plays a key role in maintaining whole-body glucose homeostasis. Early studies using the AMPK activators AICAR and metformin demonstrated inhibition of hepatic gluconeogenesis, but these effects are likely not solely AMPK mediated.[27] AICAR is a potent inhibitor of the essential gluconeogenic enzyme fructose-1,6-bisphosphatase (FBPase). Moreover, inhibition of gluconeogenesis by metformin is manifest in livers totally lacking AMPK.[28,29] However, hepatic overexpression of a constitutively active AMPK α2-subunit led to plasma glucose lowering in several mouse models and decreased key gluconeogenic gene expression.[30] Conversely, hepatic deletion of the AMPK α2-subunit results in fasting hyperglycemia.[31] AMPK activation represses gluconeogenic gene expression via

phosphorylation and cytosolic sequestration of CREB-regulated transcriptional coactivator 2 (CRTC2).[32]

4. THERAPEUTIC POTENTIAL OF AMPK ACTIVATION

A compelling case can be made for the therapeutic potential of AMPK activation for a variety of diseases. AMPK-mediated alterations in lipid metabolism should lead to reductions in whole-body lipid content. Ectopic (nonadipose) lipid deposits, particularly those in liver, muscle, and beta cells, lead to increased inflammation, lipotoxicity, and insulin resistance and, as such, contribute significantly to metabolic diseases.[33–35] Numerous AMPK activators have been shown to improve hepatosteatosis, including metformin and AICAR.[8,29,36,37] AMPK activation also modulates cardiomyocyte, smooth muscle cell, and endothelial cell function. Combined lipid effects are anticipated to improve plasma dyslipidemia, pathological lipoprotein metabolism, and atherosclerosis, thus providing major cardiovascular benefits.[38–42]

AMPK activation modulates adipocyte metabolism.[9] The enzyme inhibits and activates adipogenesis in white and brown fat cells, respectively, and the mechanism is of interest for antiobesity therapy.[14,43,44] In fact, AMPK appears to be a key mediator of the positive metabolic benefits of calorie restriction, which includes increased lifespan.[45,46] Given the proinflammatory nature of white adipose tissue, AMPK activation has potential applications in numerous inflammatory diseases.[47–49]

A major therapeutic focus for the application of AMPK activators is insulin resistance and T2DM.[50] The approved antihyperglycemic agents metformin, rosiglitazone, and pioglitazone, and the insulin sensitizing hormone adiponectin, activate AMPK both *in vitro* and *in vivo*.[51] T2DM is characterized by excessive hepatic glucose production, extreme insulin resistance resulting in reduced peripheral glucose uptake and utilization, and insufficient insulin secretion to reverse the hyperglycemia. Redressing these imbalances are among the most prominent effects of exercise contributing to glucose homeostasis. Exercise activates AMPK, and conversely, chemical activation of AMPK may mimic many of the effects of exercise, which has profound benefits in T2DM patients.[18] In addition to the direct reduction in plasma glucose, AMPK activation should also improve insulin sensitivity over time by reducing ectopic fat depots (see above).

Table 10.1 Some additional therapeutic indications for AMPK activators

Indication	References
Hypertension	52,53
Heart failure and ischemic injury	38–42, 58–60
Kidney disease	61
Osteoporosis	62
Cancer	54

Finally, AMPK activation is of interest for a variety of other pathological conditions. One such area is the treatment of hypertension. AMPK phosphorylates and activates endothelial nitric oxide synthase and also decreases vascular smooth muscle cell contractility.[52,53] An additional area to highlight is the treatment of various cancers, which has become a major focus of AMPK research over the past several years.[54] This is based largely on the tumor suppressor function of LKB1 and the well-established modulation of mammalian target of rapamycin complex 1 (mTORC1) and the p53 tumor suppressor by AMPK.[12,55–57] Other indications are listed in Table 10.1.

Importantly, the number of adverse effects as a consequence of AMPK activation is anticipated to be relatively low. Data convincingly demonstrate that activation of AMPK in the brain, by either chemical or genetic means, increases food intake.[63,64] The hormones leptin and ghrelin control food intake at least in part by modulating hypothalamic AMPK activity. Other functions of AMPK in brain, including its impact on Alzheimer's disease, are the subject of current investigation.[65,66] Another potential adverse effect involves the heart. Mutations in the γ2-subunit are found in familial Wolff–Parkinson–White syndrome patients (5′-AMP-activated protein kinase subunit γ2 (PRKAG2) cardiomyopathy).[67,68] Although somewhat controversial, these mutations appear to result in (at least intrinsic) activation of AMPK, presumably by ablating the binding of inhibitory ATP. While it remains to be established which of the clinical manifestations of PRKAG2 cardiomyopathy have developmental origins, cardiac safety is likely to be a major consideration in the development of compounds that activate AMPK in the heart.

5. PHARMACOLOGICAL AMPK ACTIVATORS

The central role that AMPK plays in glucose and lipid metabolism has aroused interest in developing pharmacological activators. This interest has been fueled by the possibility (discussed above) that AMPK activation may mediate some of the beneficial effects of exercise and calorie restriction and that it may contribute to the efficacies of certain currently used antidiabetic agents.[69] Despite this growing attention and an increase in the number of reported AMPK activators, the chemical space remains relatively narrow.

The endogenous function of AMPK as a stress-activated kinase needs to be considered when assessing reports in the area. Compounds that exhibit cytotoxicity or interact with pathways associated with cellular stressors (e.g., hypoxia, oxidative stress, etc.) may activate the enzyme indirectly through elevation of cellular AMP or calcium levels. This complicates interpretation of the mechanism underlying the effects of a particular pharmacological agent.

This section will provide a brief overview of the more important and widely reported indirect AMPK activators and a closer focus on disclosed direct AMPK activators.

Since a number of different assays are used to assess AMPK activity, structures with data refer to the following: (a) activation of unspecified AMPK complex, (b) activation of recombinant $\alpha 1\beta 1\gamma 1$ AMPK complex, (c) activation of recombinant $\alpha 1\beta 1\gamma 1$ and $\alpha 2\beta 1\gamma 1$ AMPK complex, and (d) inhibition of cellular fatty acid synthesis.

5.1. Indirect AMPK activators

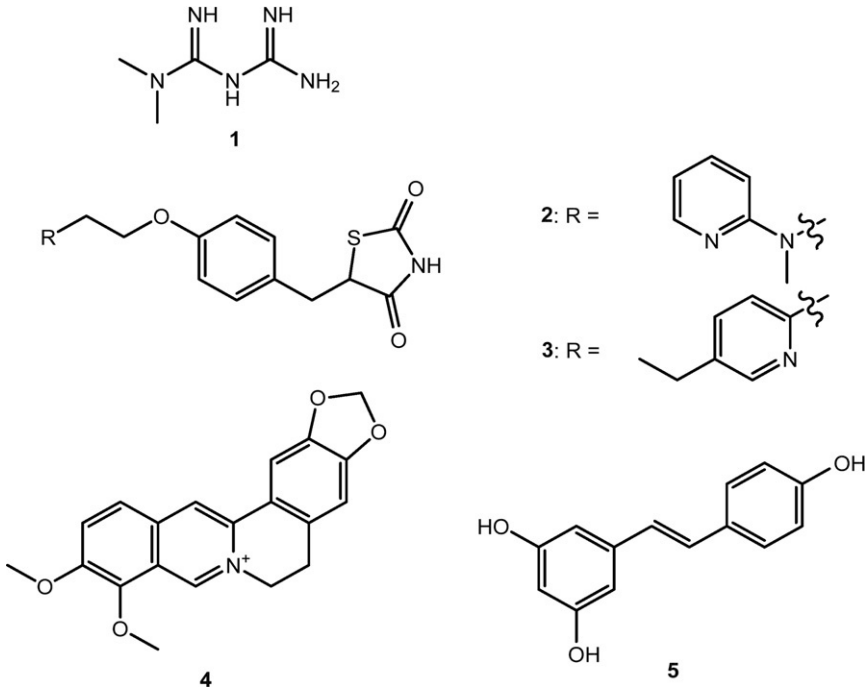
Metformin (**1**) is the first line of therapy for T2DM in most countries and it has been shown to be an LKB1-dependent indirect activator of AMPK both *in vitro* and *in vivo*.[70,71] The compound inhibits complex I of the mitochondrial respiratory chain with subsequent reduction in cellular energy charge.[29,72]

The degree to which AMPK activation contributes to the hypoglycemic efficacy of metformin is unknown. Recent data provide strong evidence that at least some effects on glucose homeostasis are AMPK-independent.[28]

The thiazolidinediones (TZDs), exemplified by rosiglitazone (**2**) and pioglitazone (**3**), are purported to have dual mechanisms of action.[73] The compounds exert transcriptional effects following PPARγ activation which result in adipogenesis, adiponectin release and reduced hepatic

gluconeogenesis.[51] In addition, *in vitro* studies and studies in mice deficient in adiponectin suggest that the compounds exert adiponectin-independent effects on skeletal muscle glucose uptake coincident with increases in AMPK and ACC phosphorylation. While the mechanism for AMPK activation has yet to be fully determined, similar to metformin, it may involve inhibition of respiratory complex I.[74]

There are numerous reports of compounds derived from natural products that activate AMPK. Of these, the alkaloid berberine (**4**) and the polyphenol resveratrol (**5**) have been most widely investigated. The mechanism of AMPK activation for both is understood to be indirect. Berberine has been shown to inhibit mitochondrial respiratory complex I in much the same fashion as metformin and the TZDs.[75] Resveratrol may reduce ATP synthesis via inhibition of F_1-ATPase.[76]

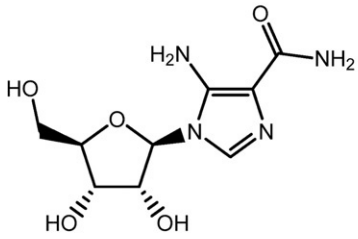


5.2. Direct AMPK activators

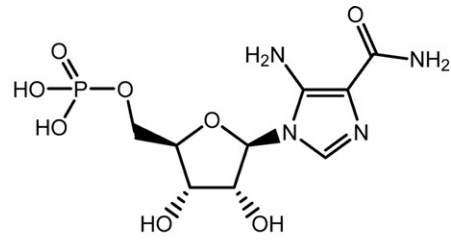
5.2.1 Synthetic nucleoside AMPK activators

Analogs of AMP have found wide use in the field as tools to investigate the effects of AMPK activation. AICAR (**6**) is a cell permeable nucleoside that undergoes intracellular phosphorylation to generate ZMP (**7**).[77] In various

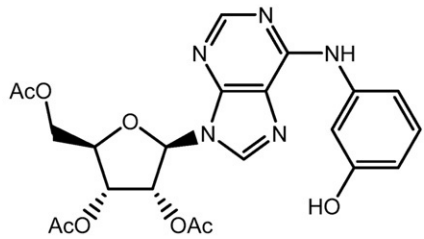
dysmetabolic rodent models, treatment with AICAR results in improvements in metabolic parameters.[78] Limitations of the compounds focus on their short half-lives and extensive off-target activities. In particular, ZMP inhibits FBPase and activates glycogen phosphorylase (GPPase)



6: IC_{50} ~ 100 μM[d]



7: EC_{50} = 164 μM[a]



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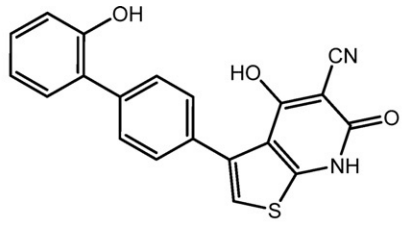
complicating interpretation of any AMPK-mediated hypoglycemic efficacy observed following AICAR administration. While poor physical properties and selectivity issues limit nucleosides as particularly promising leads for drug development, some efforts have been invested to identify new compounds. The most recently reported analog is WS010117 (**8**), which stimulates AMPK activity in HepG2 cells with consequent reductions in *de novo* lipogenesis and elevation of fatty acid oxidation. *In vivo*, the compound ($\leq$18 mg kg^{-1} QD PO, 2–8 weeks) reduced high-fat diet-induced plasma and liver lipid accumulation in hamsters.[79]

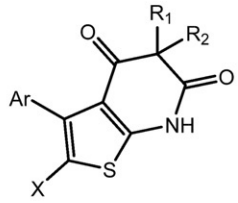
5.2.2 Nonnucleoside AMPK activators

Reports of small molecule direct AMPK activators that are not closely related to nucleosides comprise the largest area of growth in the past few years. Unfortunately, many of these reports emerge from patent applications where descriptions of data are limited. We have included generic structures and available data of disclosed series. A small number of compounds have been more widely published and we have included a broader discussion of their reported *in vitro* and *in vivo* properties.

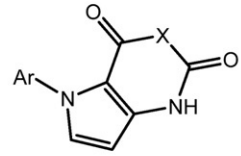
Compounds containing a pyridone core form the largest structural class in the field. The first reports described the structure and activity of a number of AMPK activators bearing a thienopyridone core.[80,81] High-throughput screening of 700,000 compounds followed by a hit-to-lead effort identified A-769662 (**9**). Since its disclosure, the compound has become an important tool in efforts to further delineate the consequences of AMPK activation.



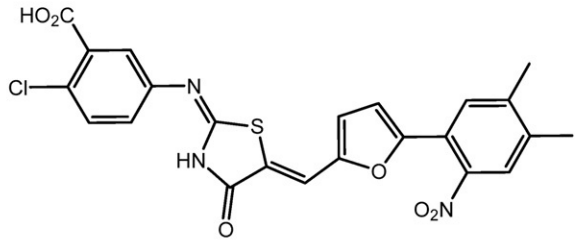
9: EC_{50} = 0.8 μM[a]



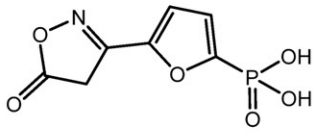
10: >75% activation vs. AMP @30 μM[a]
Ar=2-fluoro-4-methoxyphenyl;
X=Cl; R_1=Me; R_2=Ph



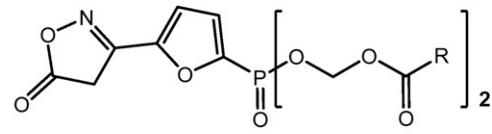
11: EC_{50} = 1.3 μM[c]
Ar=[1,1′-biphenyl]-2-ol;
X = NH



12: EC_{50} = 0.3 μM[b]



11: EC_{50} = 6.3 nM[a]



12: IC_{50} = 20 nM[d]
R=iso-propyl

A-769662 binds to a site on the enzyme distinct from the γ-subunit CBS domains and selectively activates β1-containing AMPK complexes (vs. β2-containing heterotrimers).[1] Similar to AMP, the compound is an allosteric activator of the enzyme ($EC_{50} = 0.7–0.8\ \mu M$) and additionally protects pAMPK from dephosphorylation. While off-target activities have been documented for the compound,[82] A-769662 is considerably more selective than the nucleoside analogs with minimal effect on GPPase, FBPase, and a number of other receptor, ion channel, and kinase targets.

Incubation of rat hepatocytes with A-769662 dose-dependently increased ACC phosphorylation in an AMPKK (LKB1 or CaMKKβ) and AMPK-dependent manner with consequent inhibition of lipogenesis ($IC_{50} = 3.2\ \mu M$) and without effecting intracellular adenine nucleotide levels. The compound also inhibits 3T3-L1 adipocyte differentiation.[43] At high concentrations, A-769662 induced mouse muscle glucose uptake in an AMPK-independent (P13-kinase-dependent) manner, highlighting an additional off-target activity and suggesting that activation by the compound is insufficient for AMPK-mediated glucose uptake.[83]

Due to poor oral pharmacokinetics (7% bioavailability in rats), *in vivo* study of A-769662 was conducted using IP dosing. Single acute doses ($30\ mg\ kg^{-1}$) in rats were sufficient to induce a 33% reduction in hepatic malonyl CoA levels. While fatty acid oxidation was not measured directly, the compound did cause a reduction in respiratory exchange ratio. Acute effects on hepatic gluconeogenic and lipogenic gene expression were not evident, but reductions in PEPCK (37%), G6Pase (63%), and FAS (31%) mRNA expression were observed after 5 days of dosing.

Similar to effects seen with AICAR and metformin, A-769662 ($30\ mg\ kg^{-1}$ IP BID, 5 days) reduced fed glucose levels by 30–40% with concomitant reductions in TGs in plasma (63%) and liver (48%) in *ob/ob* mice. The compound also caused significant reductions in body weight (9% after 14 days of treatment). While the body weight effects may be mechanism based, they do introduce complications in understanding the mechanisms underlying glucose and TG reductions.

Finally, recent studies suggest that AMPK activation by pretreatment with A-769662 protects the heart from ischemia–reperfusion injury in mice[84] and reduces infarct size in isolated, perfused rat hearts.[85]

A number of additional pyridones have been disclosed in patent applications. This includes a series of substituted thienopyridones (e.g., **10**) and a novel series of pyrrolopyridones (e.g., **11**). While data are limited, the

compounds appear to have similar *in vitro* potency in activating the α1β1γ1 isoform of AMPK.[86]

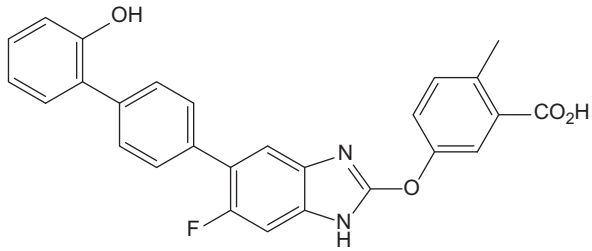
Thiazolidinone-derived PT1 (**12**) was discovered in a screen of 3600 compounds.[87] The compound likely binds to and activates the catalytic α-subunit directly via suppression of autoinhibition ($EC_{50} = 8$ μM, ~eightfold activation above baseline). Activation of the intact α1β1γ1 complex was also documented ($EC_{50} = 0.3$ μM, 1.5-fold maximal activation @ 5 μM). In cell cultures, PT1 caused a CaMKKβ-dependent increase in AMPK and ACC phosphorylation in L6 myotubes and HeLa cells without affecting the AMP:ATP ratio.

A screen of a ~1200-member library of AMP mimetics identified a hydroxy-isoxazole substituted furan phosphonic acid (**11**).[88] The compound is a full activator of human AMPK ($EC_{50} = 6.3$ nM) and a partial activator of the rat enzyme ($EC_{50} = 21$ nM, 51% of the maximal activation induced by AMP) with no activity against GPPase and FBPase and good selectivity over a panel of 64 other targets. The compound is charged at physiologic pH limiting permeability. When it was administered as an esterase-sensitive prodrug (e.g., **12**—itself unable to activate AMPK), the compound caused a significant reduction in *de novo* lipogenesis in plated rat hepatocytes ($IC_{50} = 20$ nM) concomitant with increased ACC phosphorylation. A single acute dose of compound **12** (30 mg kg^{-1} IP) was sufficient to reduce hepatic *de novo* lipogenesis by 78% over the course of 1 h in mice. Much of the SAR described focuses on prodrug design and established that sterically unencumbered phosphonate esters and carbonates maintained similar potencies in *in vitro* assays suggesting similarly rapid conversion to active compound.

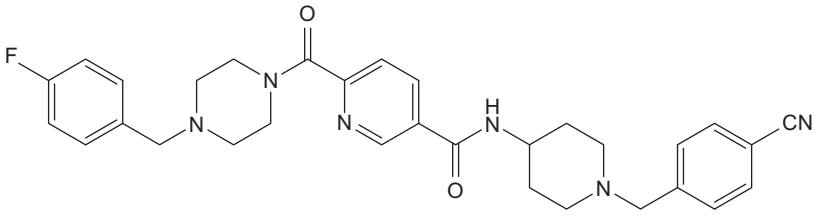
5.2.3 Other compounds

Some SAR is apparent in a series of benzimidazole compounds reported in five patent applications. Data suggest that replacement of a sulfur substituent in the 2-position of the benzimidazole with an oxygen significantly increases potency; with the latter (e.g., **13**) providing EC_{50}s in the low nanomolar range.[89]

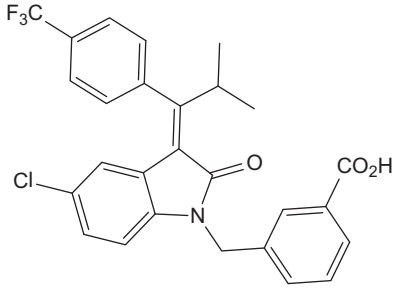
The remaining structural series have been claimed in patent applications with more limited data. This includes a series of related carboxamide, sulfonamide, and amine compounds (e.g., **14**) with potencies ranging as low as $EC_{50} < 0.1$ μM,[90] a series of oxindoles (e.g., **15** $EC_{50} = 0.66$ μM),[91] and a series of tetrahydroquinolines (e.g., **16** $EC_{50} = 1.24$ μM).[92]



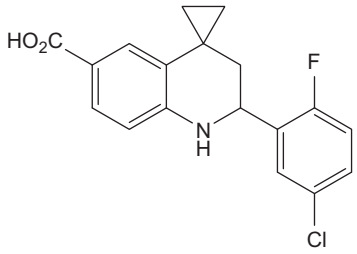
13: EC_{50} = 1 nM (Max Act = 187% vs. AMP)[a]



14: EC_{50} < 0.5 μM[a]



15: EC_{50} = 0.66 μM[c]



16: EC_{50} = 1.24 μM[c]

6. CONCLUSION

Five years ago, the development of chemotherapeutic AMPK activators would have been considered a remote possibility due to significant complexities emanating from the target's complex structure and pleiotropic function. This situation was compounded by the paucity of chemical matter capable of interacting with the enzyme. Increasing knowledge regarding the structure and mechanism of AMPK, coupled with the identification of new chemical matter in recent years, suggests that AMPK activation is indeed druggable. A significant remaining hurdle is to better understand the potential mechanism-based adverse effects of AMPK activation.

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