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Discovery and SAR development of thienopyridones: A class of small molecule AMPK activators

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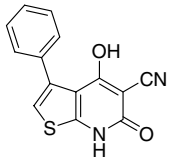
Abstract—AMP-activated protein kinase (AMPK) is well established as a sensor and regulator of intracellular and whole-body energy metabolism. A high-throughput screen was performed in order to identify chemotypes that are bound by AMPK. A novel thienopyridone compound (**1**) was identified and subsequently optimized. The structure–activity relationships that emerged from this effort are described.

Obesity and associated diseases like type 2 diabetes, the metabolic syndrome, hypertension, and atherogenic dyslipidemia represent major health risks around the world. An estimated 194 million people have either type 1 or 2 diabetes, according to the International Diabetes Federation. Type 2 diabetes is the most common and fastest growing form of the disease and is often complicated by obesity. AMPK (adenosine monophosphate-activated protein kinase), a heterotrimeric serine/threonine kinase, is well established as a key sensor and regulator of intracellular and whole-body energy metabolism.[1] Activation of AMPK alters carbohydrate and lipid metabolism to increase fatty acid oxidation and glucose uptake and decrease fatty acid and cholesterol synthesis.[2] Through its central role in the regulation of glucose and lipid metabolism, AMPK is emerging as an attractive molecular target for the treatment of diabetes, metabolic syndrome, and obesity.[2,3]

There have been a number of reports of small molecule regulators of AMPK, such as AICAR, metformin and rosiglitazone.[4] These are all indirect activators, however, and thus to further elucidate the physiological consequences of AMPK activation, our laboratory sought to identify small molecules that directly interact with this enzyme.

As part of our effort to identify and assess the effects of AMPK activation on appropriate metabolic parameters, a high-throughput screening was performed from which several structurally diverse AMPK activators were identified. This report describes the systematic evaluation of the thienopyridone agonist **1**, in addition to the structure–activity relationships (SAR) of the resultant analogs.

We initiated our efforts with a resynthesis of the HTS hit **1** (Fig. 1), followed by the preparation of several direct analogs. The synthesis of the thienopyridone compounds is outlined in Scheme 1.[5a,6] Briefly, acetophenone derivatives were treated with ethyl cyanoacetate, sulfur, and morpholine, heated at 60 °C to afford the 2-amino-4-aryl-thiophene-3-carboxylic acid ethyl esters **2**. The appropriate ethyl ester **2** was then reacted with cyanoacetic acid chloride, which was made fresh by treating cyanoacetic acid with PCl_5 in dichloromethane,



1

AMPK Rat liver EC_{50} (μM) 38

Figure 1. High-throughput screen hit: thienopyridone compound **1**.

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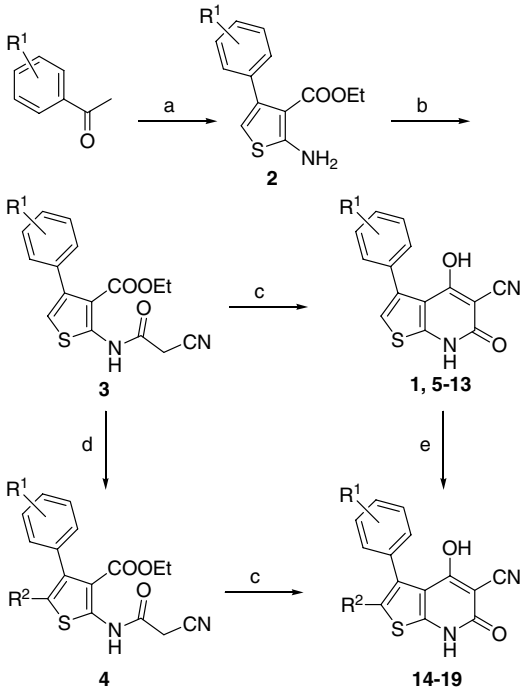
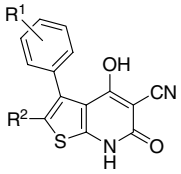


Scheme 1. Reagents and conditions: (a) $CH_2(CN)CO_2Et$, S, morpholine, EtOH, 60 °C (20–45%); (b) $CH_2(CN)CO_2H$, PCl_5, Et_3N, DCM (90%); (c) NaH, THF, reflux (80%); (d) $SOCl_2$, DCM (90%); (e) $C_5H_5NHBr_3$, AcOH or NCS/NBS, AcOH (30–60%).

in the presence of triethylamine at 60 °C to provide the 2-(2-cyano-acetylamino)-4-aryl-thiophene-3-carboxylic acid ethyl esters **3**. Following this step, the ethyl esters **3** were treated with sodium hydride in tetrahydrofuran at temperatures ranging from 25 to 80 °C to provide the final thienopyridone compounds. The final products were very polar and typically required reverse-phase HPLC purification.

Compound **1**, which showed modest AMPK activity (rat liver EC_{50}, 38 μM),[7] was our starting point for optimization (Table 1). The SAR investigation was initiated with the functionalization of the unsubstituted 3-phenyl ring. Derivatization of this ring with either a *meta*- or *para*-hydroxy group either retained or had a twofold improvement of activity of parent **1**, respectively (see **5** and **6**, Table 1). Tying these hydroxyls into a five-membered ring to produce the methylenedioxyphenyl analog **7** also improved the activity twofold, resulting in an EC_{50} of 20 μM. Other fused ring systems resulted in a diminution of AMPK activity, along with various ortho-substitutions (data not shown). Since the para-substitution position appeared to be the most productive position for increasing the potency, several more analogs with substituents at this position were prepared. Replacement of the hydroxyl group in **3** with fluoro, chloro or bromo afforded analogs that had lowered potency as compared to parent **1**. While no gain in potency was observed with the *para* ester **11**, the allyl-substituted analog **12** resulted in a compound equipotent to **1**. Finally, capping the *para*-hydroxyl moiety of **6** with a methoxymethyl group afforded the most potent analog in this group of compounds, as analog **13** showed a significantly improved EC_{50} of 8 μM.

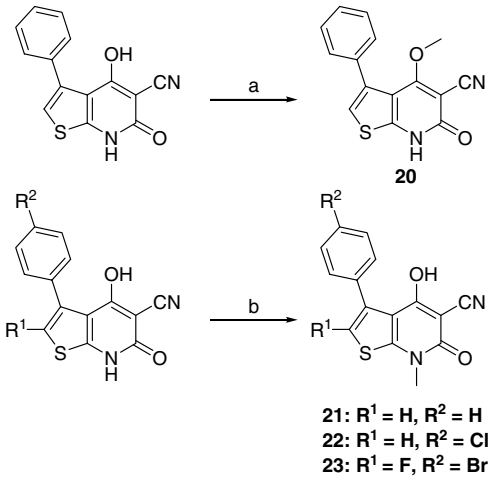
Table 1. SAR of 3-aryl thienopyridones



Compound	R^1	R^2	AMPK Rat liver EC_{50}[a] (μM)
1	H	H	38
5	3-OH	H	38
6	4-OH	H	20
7	3,4-OCH_2O	H	20
8	4-F	H	84
9	4-Cl	H	81
10	4-Br	H	72
11	4-COOMe	H	55
12	4-Allyl	H	40
13	4-OCH_2OCH_3	H	8
14	H	Cl	3.7
15	H	Br	5.8
16	4-OH	Cl	2
17	4-F	Br	10
18	H	NO_2	Inactive
19	H	2-OH-C_6H_4	Inactive

[a] All compounds were >95% pure by HPLC and characterized by ^1H NMR and HRMS. Values represent an average of at least two determinations.

Encouraged by the increase in AMPK activation afforded by the appropriately substituted phenyl analogs, we turned our efforts towards the incorporation of functionality at the 2-position of the thienopyridine core. 2-Chloro and 2-bromo substitution was effected according to Scheme 2. Briefly, 4-phenylthiophene intermediate **3** was treated with thionyl chloride in dichloromethane to afford the chlorinated intermediate **4** in 90% yield. Treatment with sodium hydride rapidly afforded the final cyclized product in good yields. Alternatively, the



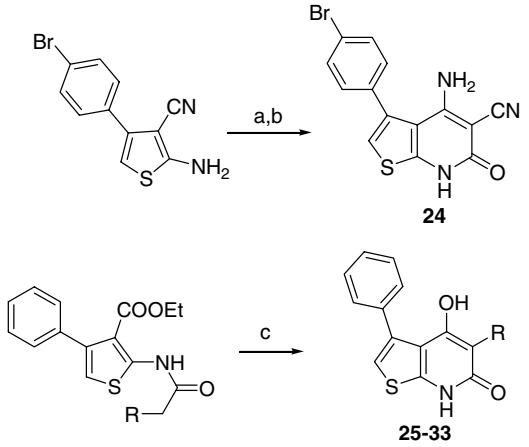
Scheme 2. Reagents and condition: (a) Diazomethane, diethyl ether (50%); (b) NaH, MeI, THF, reflux (40%).

2-chlorinated analogs could also be prepared by the treatment of analogs **1** and**6** with *N*-chlorosuccinimide, although the yields in this transformation were low (30%). The 2-brominated analogs were prepared in a straightforward manner by the treatment of analogs **1** or **8** with NBS, affording the desired products in about 60% yield.

Evaluation of these analogs in our binding assay revealed that functionalization with electron withdrawing groups at the 2-position significantly improved AMPK activation. As demonstrated in Table 1, analogs **14** and **15** had EC_{50} values of 3.7 and 5.8 μM, respectively. Compound **16**, the 2-chloro-substituted analog of **6**, showed a tenfold improvement in activity relative to the parent analog. This trend was further present in analog **17**. In contrast, incorporation of larger electron-withdrawing groups, such the nitro moiety present in **18**,[8] resulted in a loss of potency. The same result was observed with the phenyl-substituted compound **19**.[9]

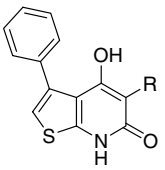
We next evaluated the requirement of the hydroxyl moiety on the thienopyridone core. To this end, the chemical reactivity of the acidic-OH and -NH were investigated. While basic alkylation conditions (sodium hydride, methyl iodide) exclusively afforded N-alkylated products, O-alkylation required more neutral conditions (CH_2N_2) for an efficient and selective transformation (Scheme 2). Finally, the amino analog **24** was prepared in an analogous manner to **1** (see Scheme 3). However, all of these modifications proved unproductive, as analogs **20**–**24** were inactive against rat liver AMPK.

Our final exploration was directed towards replacement of the 5-cyano functionality on the pyridone ring. As shown in Scheme 3, this was achieved analogously to **1** by switching from cyanoacetyl chloride to various substituted acyl chlorides in the coupling step prior to cyclization. Our attempts to generate the 5-carboxylic acid analogs were unsuccessful, as the attempted hydrolysis of the nitrile under acidic conditions only afforded the corresponding decarboxylated product **31.**



Scheme 3. Reagents and conditions: (a) $CH_2(CN)CO_2H$, PCl_5, Et_3N, DCM (90%); (b) NaOEt, EtOH, reflux (43%); (c) NaH, THF, reflux or KHMDS, THF, $PhCH_3$, 0 °C–rt, (20–70%).

Table 2. SAR of 5-substituted thienopyridones



Compound	R	AMPK Rat liver EC_{50}[a] (μM)
25	Ph	Inactive
26	3-OMe-C_6H_4	180
27	2-Thiophene	183
28	–COOMe	175
29	–NMe_2	Inactive
30	Cl	88
31	H	Inactive

[a] All compounds were >95% pure by HPLC and characterized by ^1H NMR and HRMS. Values represent an average of at least two determinations.

As shown in Table 2, only the chloride substituent could effectively replace the cyano group in the pyridone ring of **1** (analog **30**). Other substitutions resulted in a diminution or complete loss of AMPK activity up to the concentrations tested.

The systematic investigation of the HTS lead structure **1** identified several productive areas for optimization. Specifically, 3-phenyl ortho-substitution led to improvements as much as tenfold, while 2-substitution afforded analogs with improvements over fortyfold. Subsequent reports will disclose further optimization of these analogs, as well as their evaluation in in vivo models of glucose lowering.

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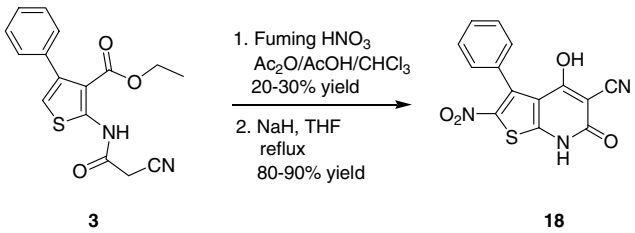
7. The rat liver EC_{50} value was measured by a 96-well AMPK assay. The AMPK activity was measured by monitoring phosphorylation of the SAMS peptide substrate (20 μM in standard assays and 100 μM in additivity assays) following a previously described protocol.[5c]

8.



3 **18**

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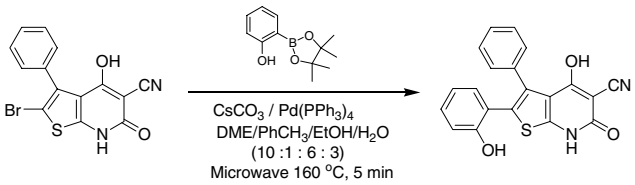

15 **19**