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2-Hydroxy-*N*-arylbenzenesulfonamides as ATP-citrate lyase inhibitors

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Abstract—A novel series of 2-hydroxy-*N*-arylbenzenesulfonamides were identified to be ATP-citrate lyase (ACL) inhibitors with compound **9** displaying potent in vitro activity (IC_{50} = 0.13 μM). Chronic oral dosing of compound **9** in high-fat fed mice lowered plasma cholesterol, triglyceride, and glucose, as well as inhibited weight gain.

Cardiovascular disease remains one of the leading causes of morbidity and mortality in developed countries with hypercholesterolemia contributing as a major risk factor.[1] Multiple clinical trials in dyslipidemic patients have shown that aggressive LDL cholesterol lowering can achieve significant reduction of coronary artery disease (CAD) events.[2] However, there is growing evidence that correlates CAD with many other independent risk factors such as diabetes, obesity, low HDL, and high triglyceride levels.[3,4] Thus, new therapeutic agents that can treat multiple risk factors continue to be an area of intensive medical research. ATP-citrate lyase (ACL) is an extramitochondrial enzyme that is expressed in lipogenic tissues such as liver and adipose.[5] Since ACL is the primary enzyme responsible for the production of cytosolic acetyl-CoA, a precursor required for de novo biosyntheses of cholesterol and fatty acids, inhibition of ACL has the potential to reduce cholesterol and triglyceride levels and possibly exert an impact on obesity via reduction of lipogenic factors.[6–8]

There are several literature reports of ACL inhibitors (Fig. 1) including (−)-hydroxycitrate (**1**) (K_i = 0.15 μM)[9] and the succinic acid derivative **2** (K_i = 1.0 μM).[10–14] However, in a HepG2 cell-based assay both compounds showed no inhibitory activity of lipid synthesis at concentrations up to 100 μM, probably due to poor cell-permeability of these polar compounds. When tested as its lactone, prodrug **3**, compound **2** exhibited 82–91% inhibition of cholesterol and fatty acid syntheses at 30 μM.[10] Oral treatment with **3** in chow fed rats also showed a decrease in plasma cholesterol and triglyceride levels.[14]

In an attempt to identify a cell-permeable ACL inhibitor, a high throughput screen of our internal compound collection was initiated. The primary goal was to iden-

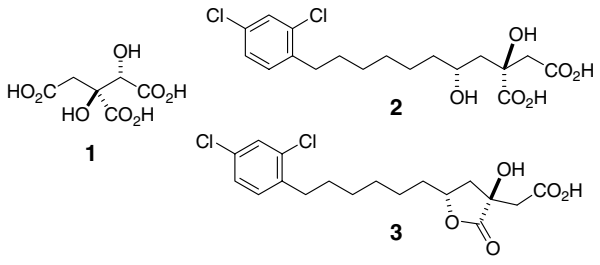

Figure 1. Examples of ACL inhibitors in the literature.

Keywords: ATP-citrate lyase (ACL) inhibitors; Lipid; Glycemic profiles and weight loss.
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tify an orally active tool compound that could be used to evaluate the efficacy potential of ACL inhibition in an animal model. Through these efforts, the 2-hydroxy-*N*-arylbenzenesulfonamide **4** was identified as a modest inhibitor of ACL (IC_{50} = 1.1 μM).[15] Subsequent similarity deck mining of the compound collection based on the 2-hydroxy-*N*-phenylbenzenesulfonamide pharmacophore identified an additional 50 analogs for testing, of which 11 showed greater than 50% inhibition at 10 μM. IC_{50} values are depicted in Table 1.[15,16] The SAR trend was unremarkable based on this limited set of compounds, with the exception of the 2-substituted anilines (i.e., compounds **8**, **9**, **13** or **14**) which appeared to be more potent than other analogs in the set. Among them, compound **9** was the most potent with an IC_{50} of 0.13 μM.[17]

In HepG2 cells, compound **9** showed inhibition of total lipid syntheses with an IC_{50} of 8 μM.[18] A cell based Alamar Blue cytotoxicity assay was used in parallel to differentiate the effect on the inhibition of lipid synthesis versus potential cytotoxicity.[19] Under identical incubation conditions, compound **9** showed no cytotoxicity up to 50 μM, indicating the observed inhibition of lipid synthesis was not a result of compound-induced cytotoxicity.

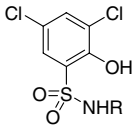
To assess if compound **9** was suitable for oral dosing, a standard pharmacokinetic assessment was performed. In mice, **9** showed an oral bioavailability of 55% but a relatively short half-life of 2.1 h.[20] We therefore decided to dose **9** admixed in the food to assure greater duration of exposure in subsequent chronic efficacy studies.

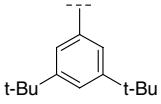
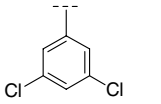
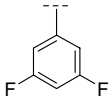
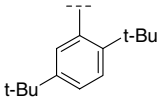
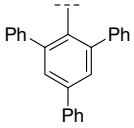
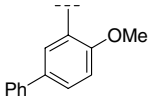
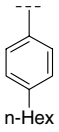
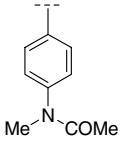
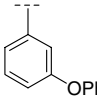
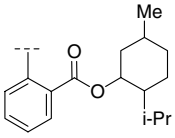
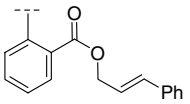
In contrast to the reported hypolipidemic effect of compound **3** in normal fed rats,[14] no clear trend for lipid lowering or hepatic lipid synthesis inhibition was observed when compound **9** was dosed chronically in mice that were fed a chow diet (data not shown). We subsequently examined the effect of **9** in high-fat fed mice, a model which more closely mimics typical western dietary intake. There were a total of four groups in the study; mice on normal diet and high-fat diet controls, and two treated groups that were supplemented with **9** in their high-fat diet to an equivalent daily dose of 10 or 100 mg/kg. The study was continued for a total of 34 days. Food consumption and body weight gain were tracked along with weekly assessment of lipid and glucose plasma chemistries[21].

As shown in Figure 2, there was a modest lowering of both plasma cholesterol and triglycerides after 20 days of treatment. A reduction in fasting plasma glucose was observed from day 7 to completion of the study. At day 29, cholesterol was lowered by 19% with the 100 mg/kg dose and triglycerides were lowered by 26% in both the 10 and 100 mg/kg treatment groups. Fasting plasma glucose was lowered by 48% and 32% for 10 and 100 mg/kg doses, respectively, as summarized in Table 2.

The high-fat diet produced a 13.8% weight gain over the course of the study while mice on normal diet for the same length of time gained 7.8% (Fig. 3 and Table 3). The 10 and 100 mg/kg treatment groups gained only 7.2% and 3.0%, respectively, from their starting weights.

Table 1. In vitro SAR summary



Compound	R	IC_{50}[a] (μM)
1		0.15
2		2.1
4		1.1
5		2.3
6		7.6
7		5.6
8		0.19
9		0.13
10		6.9
11		10.9
12		8.1
13		0.37
14		0.34

[a] In vitro data are at least two separate measurements using recombinant hACL, see Ref. 11 for detail assay condition.

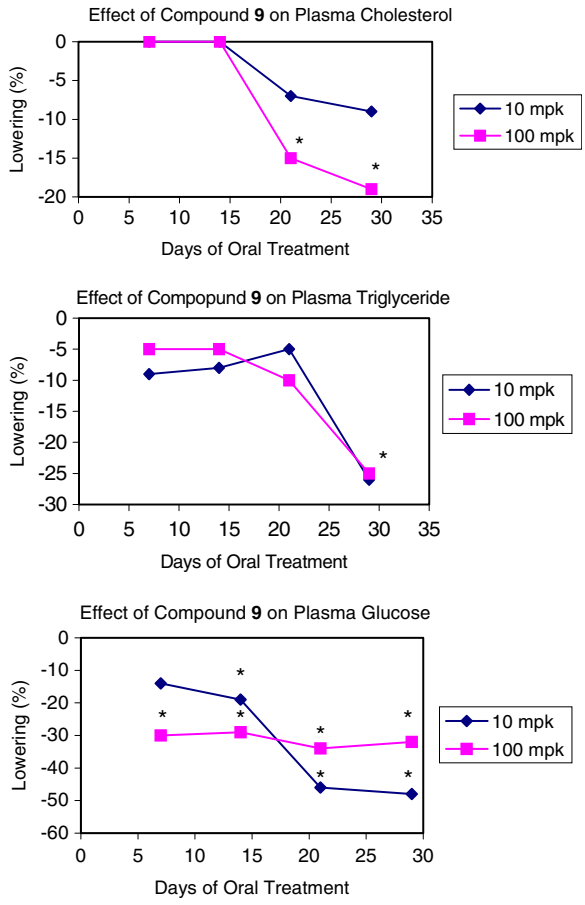


Figure 2. The reduction (%) of plasma lipids and glucose are expressed as compound **9** treated group versus high-fat fed controls. Data points with an asterisk were statistically significant ($p < 0.05$) versus non-treated high-fat fed mice.

Table 2. Effect of compound **9** on plasma lipids and glucose

Dose (mpk/day)	Cholesterol[a] (%)	Triglycerides[a] (%)	Glucose[a] (%)
10	−9[b]	−26	−48
100	−19	−26	−32

[a] Percent reduction versus high-fat diet control group as measured at day 29.
[b] Not statistically significant ($p > 0.05$); all other values were statistically significant versus high-fat fed control group ($p < 0.05$).

Relative to high-fat fed controls, treatment with 10 mg/kg of **9** showed a trend toward decreased weight gain, whereas it became significant at the 100 mg/kg dose (Table 3). Importantly, no apparent changes in food consumption among high-fat fed groups were observed,[21] indicating that the reduction in body weight gain and lipid/glucose parameters was not attributed simply to a difference in food intake between the treated groups. Additionally, no overt toxicities, including changes in locomotor activity, plasma transaminases or liver composition, were observed in any group (data not shown).

Body composition was determined by DEXA analysis at the completion of the study (Fig. 4 and Table 3). As expected, mice on the high-fat diet exhibited a significant

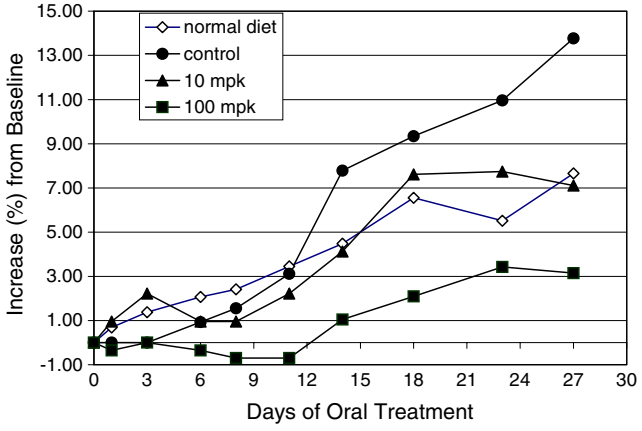


Figure 3. Effects of compound **9** on body weight.

Table 3. Effect of compound **9** on body weight and fat

Dose	Body weight gain[a] (%)	Fat % as whole body[b]
Normal diet	7.8 ± 1.2	13.7 ± 1.3
High-fat diet	13.8 ± 1.5	25.4 ± 3.4
High-fat diet + 10 mpk/day	7.2 ± 2.5[c]	12.9 ± 1.6[c]
High-fat diet + 100 mpk/day	3.0 ± 0.7[d]	7.3 ± 1.1[d]

[a] Percent (%) of body weight gain ±SEM for day 27 versus day 0.
[b] Percent (%) ±SEM, DEXA analysis was performed at day 34.
[c] Not statistically significant ($p > 0.05$) versus high-fat diet group.
[d] Statistically significant ($p < 0.05$) versus high-fat diet group.

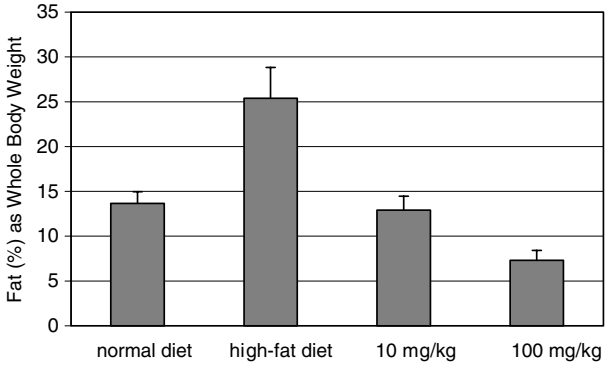


Figure 4. Fat percentage relative to whole body weight at day 34 as determined by DEXA analysis.

increase in adiposity as compared to the normal fed group (13.7% body weight as fat for normal diet vs 25.4% body weight as fat for high-fat diet group, an 85% increase; Table 3). In contrast, the 10 mg/kg treatment resulted in a comparable fat composition to the lean, normal-chow fed control, while the 100 mg/kg group experienced a 71% reduction in adiposity relative to high-fat control. The epididymal fat pads from these mice were also weighed, and the reduction in adiposity was qualitatively similar to the results from the DEXA analysis. Furthermore, a reduction of fat tissue was seen across several anatomical areas including the neck, kidney, and in the subcutaneous depot (data not shown).

In summary, we have identified a 2-hydroxy-*N*-arylbenzenesulfonamide **9** as a cell-permeable ACL inhibitor

with modest potency.[22] When administered to mice fed on a high-fat diet at 10 and 100 mg/kg/day, it produced an approximate 20–30% lowering in plasma cholesterol and triglycerides, as well as a 30–50% decrease in fasting plasma glucose. More intriguingly, chronic treatment with **9** showed a gradual inhibition of weight gain along with a reduction in adiposity without apparent changes in food intake. Using this high-fat diet mouse model, our preliminary results suggest that inhibition of ACL results in improved lipid and glycemic profiles as well as decreased adipogenesis, ultimately leading to a reduction in body weight gain. However, future studies will be required to clarify the mechanism and probe the role of ACL in the regulation of metabolic pathway and its therapeutic potential.

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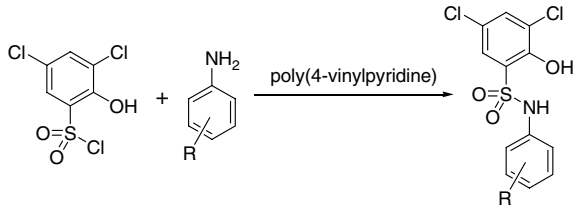
References and notes

1. Murray, C. J.; Lopez, A. D. *Lancet* **1997**, *349*, 1436.
2. (a) Gotto, A. M. *Am. J. Cardiol.* **2001**, *87*, 13B; (b) Brown, W. V. *Am. J. Cardiol.* **2001**, *87*, 23B.
3. Frick, H. M.; Elo, O.; Haapa, K., et al. *N. Engl. J. Med.* **1987**, *317*, 1237.
4. Cullen, P. *Am. J. Cardiol.* **2000**, *86*, 943.
5. Elshourbagy, N. A.; Near, J. C.; Kmetz, P. J.; Sathe, G. M.; Southan, C.; Strickler, J. E.; Gross, M.; Young, J. F.; Wells, T. N.; Groot, P. H. *J. Biol. Chem.* **1990**, *265*, 1430.
6. Srere, P. A. *Adv. Enzymol.* **1975**, *43*, 57.
7. Houston, B.; Nimmo, H. G. *Biochem. J.* **1984**, *224*, 437.
8. Groot, P. H.; Pearce, N. J.; Gribble, A. D. *Curr. Med. Chem.—Immunol., Endocr. Metab. Agents* **2003**, *3*, 211.
9. Sullivan, A. C.; Singh, M.; Srere, P. A.; Glusker, J. P. *J. Biol. Chem.* **1977**, *252*, 7583.
10. Gribble, A. D.; Ife, R. J.; Shaw, A.; McNair, D. J.; Novelli, C. E.; Bakewell, S.; Shah, V. P.; Dolle, R. E.; Groot, P. H.; Pearce, N.; Yates, J.; Tew, D.; Boyd, H.; Ashman, S.; Eggleston, D. S., et al. *J. Med. Chem.* **1998**, *41*, 3582.
11. Gribble, A. D.; Dolle, R. E.; Shaw, A.; McNair, D.; Novelli, R.; Novelli, C. E.; Slingsby, B. P.; Shah, V. P.; Tew, D.; Saxty, B. A.; Allen, M.; Groot, P. H.; Pearce, N.; Yates, J. *J. Med. Chem.* **1996**, *39*, 3569.
12. Dolle, R. E.; Gribble, A.; Wilks, T.; Kruse, L. I.; Eggleston, D.; Saxty, B. A.; Wells, T. N. C.; Groot, P. H. E. *J. Med. Chem.* **1995**, *38*, 537.
13. Dolle, R. E.; McNair, D.; Hughes, J.; Kruse, L. I.; Eggleston, D.; Saxty, B. A.; Wells, T. N. C.; Groot, P. H. E. *J. Med. Chem.* **1992**, *35*, 4875.
14. Pearce, N. J.; Yates, J.; Berkhout, T. A.; Jackson, B.; Tew, D.; Boyd, H.; Camilleri, P.; Sweeney, P.; Gribble, A. D.; Shaw, A.; Groot, P. H. *Biochem. J.* **1998**, *334*, 113.
15. The in vitro enzyme assay was performed using recombinant human ACL, as described in Ref. 11.
16. General synthetic scheme for the preparation of 2-hydroxy-*N*-phenylbenzenesulfonamides **4**–**14** is described herein.



17. Compound **9**. ^{1}H NMR ($CDCl_3$) δ 8.66 (br s, 1H), 7.68 (d, J = 2.2 Hz, 1 H), 7.30–7.55 (m, 8H), 7.20 (br s, 1H), 6.84 (d, J = 8.5 Hz, 1H), 3.71 (s, 3H); LC/MS m/z = 422 (M−H); Anal. $C_{19}H_{15}Cl_2NO_4S$: Calcd C, 53.78; H, 3.56; N, 3.30; S, 7.55; Cl, 16.71. Found: C, 53.63; H, 3.57; N, 3.17; S, 7.86; Cl, 16.62.
18. Inhibition of total lipid synthesis assay. HepG2 cells were incubated for 6 h after addition of the testing compound and [^{14}C]-alanine was added for the last 4 h of incubation. Cell lipids were extracted, separated by TLC. The incorporation of [^{14}C]-labeled in the total lipids was measured using Packard Imager.
19. Alamar Blue assay kits were purchased from BioSource Back, S. A.; Khan, R.; Gan, X.; Rosenberg, P. A.; Volpe, J. J. *J. Neurosci. Methods* **1999**, *91*, 47.
20. Pharmacokinetics study of **9**. Mice were dosed at 10 mg/kg (iv and po) in a vehicle containing 10% ethanol, 45% water, 45% PEG-400. $F\%$ = 55%, $T_{1/2}$ = 2.1 h, C_{max} = 13 μM (po), T_{max} = 0.5 h (po), total body clearance 17.3 mL/min/kg.
21. Eight-week-old C57BL/6 mice with an average 30 g of body weight were used with five mice per group. The high-fat diet contained 30% coconut oil and 45% sucrose, whereas the normal-diet group was fed with Teklad Global 18% protein rodent diet. The average food consumption for the high-fat control and **9** treated group was 3.2 ± 0.2 g/day/mouse. The average food consumption for normal diet mice was 4.0 ± 0.1 g/day/mouse.
22. In a broad screen against other metabolic disease related targets such as PTP-1B, PPAR, and ACC, it was determined that compound **9** had an IC_{50} of 6 μM and 12 μM against human ACC1 and ACC2, respectively. While it is unlikely that the in vivo efficacy of **9** in the high-fat feeding model was the result of ACC inhibition, contribution of this mechanism or other potential off-target mechanisms in the pharmacological response cannot be completely ruled out.