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Long hydrocarbon chain diols and diacids with central ether or ketone moieties that favorably alter lipid disorders

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Long hydrocarbon chain derivatives with *bis*-terminal hydroxyl or carboxyl groups and various central moieties (ketone, ether, ester, amide, carbamate, etc.) have been synthesized and evaluated for their effects on the *de novo* incorporation of radiolabeled acetate into lipids in primary cultures of rat hepatocytes as well as for their effects on lipid, glycemic and body weight variables in female obese Zucker fatty rats following one and two weeks of oral administration. The most active compounds were found to be symmetrical with four to five methylene groups separating the ether or ketone central functionality from the *gem* dimethyl, cycloalkyl or methyl/aryl substituents. Cycloalkyl substitution α to the carboxyl group in keto-acids lowered the *in vitro* activity to micromolar values. Furthermore, *in vivo* biological activity was found to be greatest for cyclopropyl-substituted ketone derivatives, particularly the ketodiacid with five methylene groups on each side of the central ketone functionality, which was identified as an HDL elevator and was also found to reduce insulin and glucose.

1. Introduction

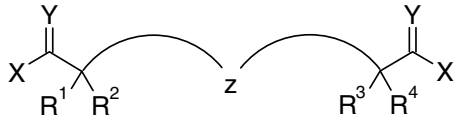
The metabolic syndrome represents one of the growing public health problems in the industrialized and more recently in economically emerging countries. Epidemiological studies have demonstrated that elevated serum cholesterol levels, particularly high values of low-density lipoprotein cholesterol (LDL-C) as well as elevated blood triglyceride (TG) levels (Gotto 1998; Sprecher 1998; Assmann et al. 1998), are correlated with a higher incidence in coronary artery disease (CAD) (Hubert et al. 1987; Gotto 2002) in the human population, while high values of high-density lipoprotein cholesterol (HDL-C) levels lower the risk of developing CAD (Luc 2002).

In the past forty years efforts in the pharmaceutical industry have been directed at identifying pharmacologic agents that can alter blood lipid levels, starting with the discovery of the hypolipidemic activity of clofibrate in the 1960s, followed by other hypolipidemic fibrates including bezafibrate, ciprofibrate, fenofibrate, and gemfibrozil in the 1970s.

A breakthrough in the field was brought in the late 1970s and 1980s by the discovery of the statin class of drugs targeted at inhibiting HMG-CoA reductase, which was possible due to the elucidation of the cholesterol *de novo* synthetic pathway (Bloch 1965), coupled with the discovery of the low density lipoprotein receptor and the metabolic basis of its regulation (Brown and Goldstein 1986). Statins markedly reduce low density lipoprotein cholesterol (LDL-C) and morbidity and mortality in humans as evidenced in both primary (Shepherd et al. 1995; Downs et al. 1998) and secondary (Scandinavian Simvastatin Survival Study (4S), 1994; Sacks et al. 1996; The Long-term Intervention with Pravastatin in Ischaemic disease (LIPID), 1998) prevention trials. The Helsinki Heart (Frick et al. 1987) and the VA HIT (Rubins et al. 2001; Robins et al. 2001) trials showed gemfibrozil's triglyceride (TG) and LDL-C reduction and modest increases in serum high density lipoprotein cholesterol (HDL-C) levels to be significantly associated with reduction in secondary coronary events and stroke.

While the fibrate and statin market is well established nowadays, there still remains an additional need for therapeutic agents with novel mechanisms for the treatment and management of isolated and mixed dyslipidemia. Much can be learned from SAR studies of new agents, including whether structural differences either enhance, lessen or do not alter biological activity, in conjunction with compound properties that influence ADME and drug safety. These properties can only be realized through *in vivo* evaluation in appropriate models and can influence the discovery and development of new agents. Thus, there is a need to discover additional agents, albeit not necessarily the most potent, but rather the best combination of biological effects and safety. The assessment of these properties for the differing structures allows a selection of desirable candidate compounds.

Some groups considered a biochemical design based on the idea that by mimicking long-chain fatty acids one can inhibit lipid synthesis along with raising the HDL-C levels. For example, Sircar et al. (1983) described a series



I

Spacer groups: symmetrical and unsymmetrical alkyl chains, aryls
Z (central groups): C=O, O, S, S=O, S(=O)$_2$, NH, N(OH), NHCONH, NHSCNH, CH(OH), OP(=O)(OH)O, NHC(=O)O, etc.
R^1 to R^4: Me, Ph, -C_6H_4-Alk, cycloalkyl (C_3–C_6)
X, Y (end groups): [HO, H_2 (alcohols)]; [HO, O (carboxylic acids)]; [RNH-, O (amides)]; [$(HO)_2P(O)O$-, H_2 (phosphates)]; etc.

of phenylenebis(oxy)bis[2,2,-dimethylpentanoic acid]s that blunted the HDL-C reduction induced in rats fed a peanut oil, cholesterol, and cholic acid containing diet. Bar-Tana et al. (1989, 1999) designed nonmetabolizable long-chain fatty acid analogues as potential hypolipidemic agents called "MEDICA" for ββ′-**me**thyl-α,ω-**dic**arboxylic **a**cids. The lead compound 3,3,14,14-tetramethylhexadecanedioic acid (MEDICA 16) was selected for clinical trials. Berge et al. (1989) have proposed a series of 3-thia fatty acids, while Bisgaier et al. have developed and studied gemcabene, a dialkyl ether dicarboxylic acid that significantly raised HDL-C in rats in contrast to MEDICA 16 and 3-thia fatty acid compounds that tend to lower HDL-C or HDL-associated proteins in that species (Bisgaier et al. 1998a,b). However, in clinical trials the effect of gemcabene on serum HDL-C and TG levels was shown to be dependent upon baseline TG levels and dose in human subjects with low levels of HDL-C (Bays et al. 2003).

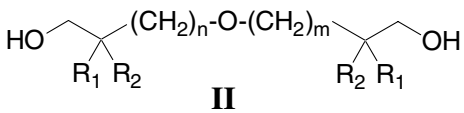
Continuing this approach, we rationally designed fatty-acid-like structures with various central moieties having diverse stereochemistry, chemical behavior and topology, with the goal of examining the influence of those significant structural modifications on the ability to favorably alter lipid disorders and to aid in the selection of a desirable compound for further development (Oniciu et al. 2005).

Our efforts in the discovery of molecules with lipid-lowering activity have been focused on the following objectives: (i) lipid- and cholesterol-lowering activity; (ii) high-density lipoprotein cholesterol (HDL-C) elevation; (iii) antidiabetic and antiobesity properties; (iv) good tolerance in an *in vivo* safety model; (v) prevention of atherosclerotic plaque progression; and (vi) promotion of atherosclerotic plaque regression.

To date there is no single, specific molecular target positively identified to be responsible for the activity of long-chain hydrocarbon compounds in general (Bar-Tana et al. 1985; Bisgaier et al. 1998c; Elokdah et al. 2004). Therefore, their lipid regulating activity was explained by cumulative, maybe synergistic effects on multiple targets. For this reason, biological activity was tested in cell-based as well as in animal models in order to ensure a full complement of operative biochemical pathways.

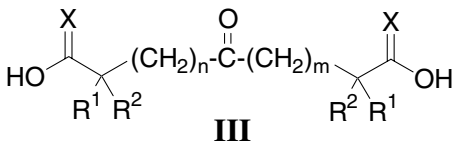
We have first explored the activity of the most synthetically accessible targets bearing four- and five-hydrocarbon or heterocarbon chain symmetrical spacers, tetraalkyl substitutions next to the terminal functionality, and central groups such as ether, ketone, sulfide, sulfoxide, sulfone, hydroxymethylene, amine, hydroxylamine, ester, amide, phosphate, carbamate, urea, thiourea etc. (structure I, Oniciu et al. 2005).

We have further discovered that the most potent compounds are those with terminal carboxyl and hydroxymethylene groups, and ethers or ketones as central moieties. Consequently, we have selected these structures as



II

n, m = 3 – 5; R_1, R_2 = CH_3, Ph, cyclopropyl to cycloheptyl; and related structures



III

X = O, H_2, n, m = 3 – 7; R_1, R_2 = CH_3, Ph, 4-$CH_3C_6H_4$, 4-iBuC_6H, cyclopropyl to cycloheptyl, and related structures

hits. To determine a lead compound, we have further investigated series of ether- and ketodiols and diacids of general formulae II and III, and examined the influence of chain length, aromatic rings, symmetry, terminal groups, and substitution pattern at the quaternary carbons α to the terminal carboxyl or β to the hydroxyl moieties (tetraalkyl, bis(alkyl-aryl) and cycloalkyl).

The cell-based assay utilized for compound screening relied on the inhibition of lipid synthesis in primary cultures of rat hepatocytes. We have used as an animal model for *in vivo* screening the female Obese Zucker rat, which is a genetically induced hyperphagic model of human non-insulin dependent diabetes mellitus. This animal has a mutation in the leptin receptor, which produces an age-dependent disease progression observed in the following parameters: hypertriglyceridemia, increased very-low-density lipoprotein cholesterol (VLDL-C), decreased HDL-C, hyperinsulinemia and obesity.

2. Investigations, results and discussion

Characteristic for all of the molecules investigated for lead compound selection were the central ether and ketone moieties connected via two linear carbon spacers to the terminating acids or in some cases alcohols, which differed in their pattern of a-substitution. Ether derivatives were synthesized by various modifications of the Williamson synthesis (Dasseux and Oniciu 2002a, b; Mueller et al. 2004a). The key step in the syntheses of most of the ketones was the alkylation of the formaldehyde synthon tosylmethyl isocyanide (TosMIC) with a properly functionalized halo-ester (Dasseux and Oniciu 2002a,b; Mueller et al. 2004a; Mueller et al. 2004b; Bell et al. 2005). We have prepared ketone derivatives with tetraalkyl and bis(alkyl-aryl) *gem*-substitution (Dasseux and Oniciu 2004a, Mueller et al. 2004b), and ketone derivatives with cycloalkyl *gem*-substitution (Dasseux and Oniciu 2004b, Bell et al. 2005).

2.1. In vitro measurement of lipid synthesis in isolated hepatocytes

The compounds were tested for inhibition of lipid synthesis in primary cultures of rat hepatocytes (Mueller et al. 2004a; Mueller et al. 2004b; Bell et al. 2005). Rat hepatocytes from male Sprague-Dawley rats were isolated essentially as described by the method of Seglen (Seglen 1979).

2.2. In vivo effects on lipid variables in female Obese Zucker fatty rats

Biological parameters were obtained following experiments performed in ten- to twelve-week old (400–500 g) female obese Zucker fatty rats Crl: (Zuc)-faBR (Mueller et al. 2004a; Mueller et al. 2004b; Bell et al. 2005), which were administered test agents or vehicle for two weeks. The animal model was first characterized to determine the age-dependent changes in body weight, serum triglycerides and the ratio of HDL-C to VLDL-C plus LDL-C (non-HDL cholesterol) in male and female Obese Zucker rats on a chow diet alone (Fig. 1). Serum lipoprotein cholesterol levels were determined by lipoprotein profile analysis. Lipoprotein profiles were analyzed using gel-filtration chromatography on a Superose 6HR (1×30 cm) column equipped with on-line detection of total cholesterol as described by Kieft (Kieft et al. 1991). The total cholesterol content of each lipoprotein was calculated by multiplying the independent values determined for serum total cholesterol by the percent area of each lipoprotein in the profile.

Our structure optimization was performed *in vivo*, because multiple mechanisms of action (MOAs) may impact the biological activity in such a complex biological system as the whole animal. This behavior is not unusual in compounds presenting HDL-C-elevating properties in various animal models (Elokdah et al. 2004). Unlike hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), our compounds inhibit both fatty acid and cholesterol syntheses in cultured liver cells at micromolar concentrations, and also increase fatty acid oxidation (Bisgaier et al. 1997, Cramer et al. 2004). In this way they reduce the availability of lipids for triglyceride (TG) synthesis and very-low-density lipoprotein (VLDL) assembly.

We have performed multiple experiments on this class of compounds (with terminal cycloalkyl and dimethyl substitution) and have determined that a major mode of action (within minutes of dosing) was inhibition of fatty acid synthesis (FAS) at the acetyl-coenzyme A-carboxylase (ACC) step *via* an allosteric mechanism (Cramer et al. 2004).

The inhibition of fatty acid synthesis produced by such an MOA was consistent with the lowering of serum triglycerides. The compounds were dual inhibitors of lipid synthesis as they rapidly block *de novo* cholesterol synthesis at a step between formation of acetoacetyl-coenzyme A and HMG-CoA (Cramer et al. 2004).

Representations of the biochemical pathways for glycolysis, fatty acid oxidation and lipogenesis are shown in Fig. 2.

The integration of lipolysis and lipogenesis is orchestrated through the ACC/malonyl-coenzyme A/carnitine palmitoyltransferase I (CPT-I) axis (McGarry 1978, McGarry 1979) whereby increased concentrations of malonyl-CoA during lipogenesis inhibit fatty acid transport into the mitochondria at CPT-I and decrease oxidation of fatty acids. The use of acetate, which is readily taken up into the cell, enables incorporation of radiolabeled acetate into substrates for both fatty acids and sterols (cholesterol) as indicated in Fig. 2.

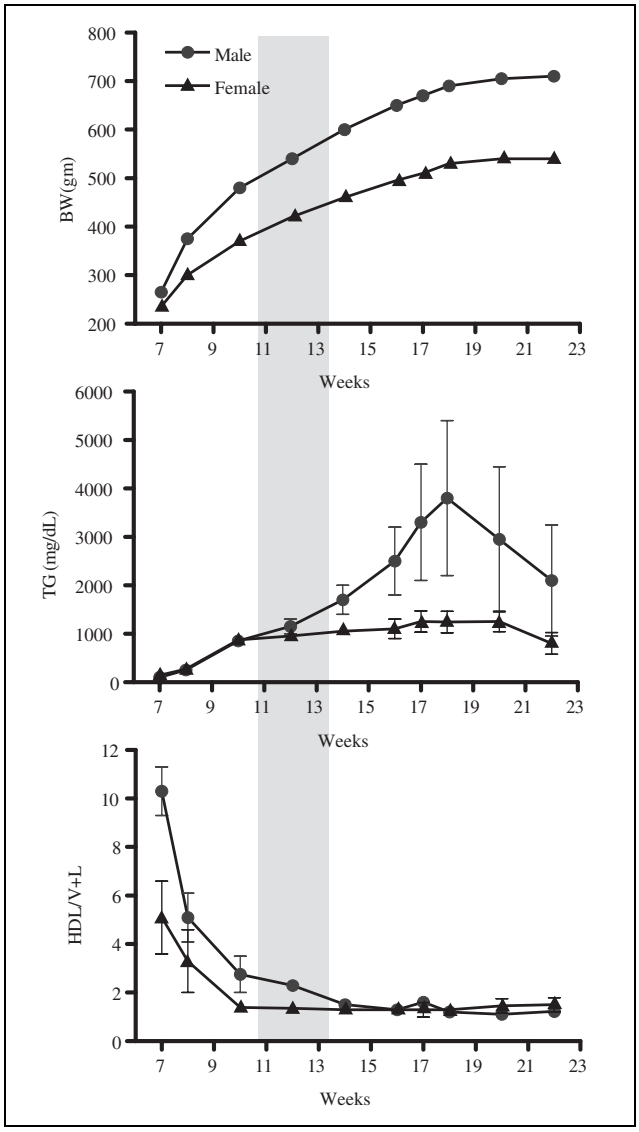


Fig. 1: The Obese Zucker Fatty rat has a dysfunctional leptin receptor and is characterized by development of age-dependent hypertriglyceridema, increased levels of VLDL-C, decreased HDL-C, hyperinsulinemia, hyperphagia, and obesity. This animal is a genetic model of non-insulin dependent diabetes mellitus (Type 2 diabetes). In our experiments, we assess lipid content (triglycerides and cholesterol) and cholesterol distribution among lipoproteins prior to and following (one-week and two-weeks) administration of test agents or vehicle in blood serum in 10–12 week old female animals (at study start). In this progressive disease model, baseline serum values are highly variable between animals, and therefore each animal is compared to its own baseline value. To minimize variation experiments are performed in the tenth to twelfth week of age. The figure shows changes in body weight, serum triglycerides, and the ratio of HDL-C to VLDL-C plus LDL-C in both male and females without treatment on a normal rodent chow diet

2.3. Activity of the compounds

Table 1 displays selected *in vitro* results for studied compounds. A few examples of compounds with three methylene spacers on each side of the central ether or ketone moieties were inactive or showed only marginal activity; therefore they were not pursued further (Dasseux and Oniciu 2002a; Mueller et al. 2004a).

The most potent compounds belonged to the cycloalkyl series (compounds **3**–**5**) and were micromolar inhibitors *in vitro* of both fatty acid and cholesterol biosynthesis (Bell et al. 2005).

Compounds with methylene spacer chain lengths of four and five and *gem*-dimethyl substitution showed good activities in both ether (**12**, **14** and **19**) and ketone series (**8**–**11**) (i.e., IC_{50}s, 2–17 μM), while compounds with six or seven methylene spacers (**16**, **18**) showed inhibition in the upper range (i.e., IC_{50}s, 9–14 μM). Compounds such as

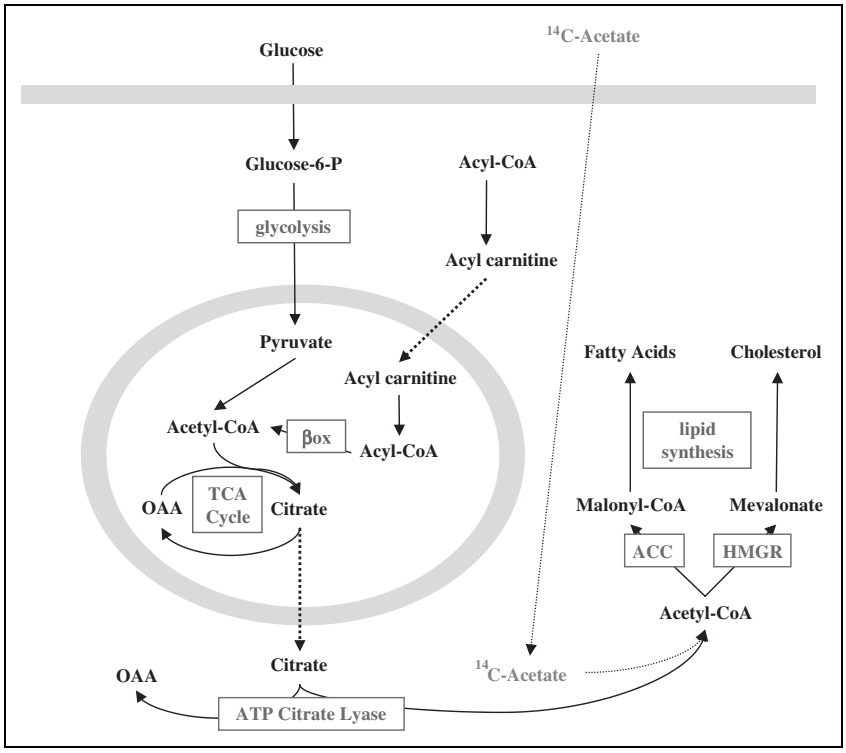


Fig. 2:
Glucose and Lipid Metabolism: Hepatocyte Lipid Synthesis Screen. Abbreviations: Box, beta-oxidation; OAA, oxaloacetate; TCA, tricarboxylic acid; ACC, acetyl-coenzyme A carboxylase; HMG-R, hydroxymethyl-coenzyme A reductase, Acyl-coenzyme A

benzophenone **23** and ether **24** with similar topological distances between the terminal groups and the central ether and ketone moieties as their aliphatic congeners displayed a significantly reduced lipid synthesis inhibitory activity (IC_{50}, 42 and 53 μM, respectively). Unsymmetrical compounds, such as **10** and **20**, possessing chains of three to five methylene spacer groups flanking the central ether and ketone showed activities similar to the symmetrical compounds with two chains of four or five methylene spacers (compounds **8**, **9**, **19**). As for the *gem* substitutions, the addition of a single phenyl substituent to one of the *gem* sites on the quaternary carbon (**14**) did not markedly change the activity (IC_{50}, 17 μM) compared to the tetramethyl-substituted ketodiol (**19**, IC_{50}, 11 μM), while substitution of one of the methyl groups with aryl groups on both quaternary carbons markedly reduced activities compared to the corresponding tetramethyl-substituted compounds and made the compounds inactive (e.g., compound **2**). Examples have been discussed in detail earlier (Mueller et al. 2004a, 2004b)

Regarding the terminal functional groups, there was no significant difference in the *in vitro* activity between diacids or diols (e.g., **10** vs **11**), which was further explained by the metabolic pathway of diols that involves transformation into the corresponding diacid as the first step (unpublished results of the same group).

Table 2 presents *in vivo* lipid variables in female Obese Zucker fatty rats. It is well known that this model has been used for the discovery and development of anti-diabetic glitazone compounds. Glitazones also show lipid regulation (TG lowering), a biological activity that translates from the Zucker rat model to humans. In the case of the structures we have focused on, there is one similar example, PD 72953, that was fortunately tested in both species (Bays et al. 2003; Bisgaier et al. 1998a, 1998b). In regards to our compounds, only one (compound **11**) to date has been studied in human subjects for safety, but has not been tested for lipid regulation in humans. However, for PD 72953 (i.e., gemcabene), studied more extensively, appears to show a predictive value between rodent models and humans (Bays et al. 2003; Bisgaier et al. 1998a, 1998b). We previously reported on compound **19**, the diol precursor of PD 72953, which is rapidly and extensively metabolically converted to PD 72953 in the Zucker rat. This compound showed a robust lipid regulating activity in this animal model (Mueller et al. 2004a). The predictive value between rats and humans can only be determined by testing in dyslipidemic humans, and there are many developmental steps that need to be satisfied before this can occur.

In the selection of the lead candidates we relied on the *in vivo* SAR approach because many factors that influence biological activity are not present *in vitro*. In order to undergo further development, biologically active agents need to show activity in complex systems and the lack of complexity may compromise the SAR found in reduced systems. One of the advantages of our screening method was that each animal served as its own control and allowed a paired analysis of data. This is an important point since the animals developed a disease state where the baseline values were highly variable due to the rapid development of dyslipidemia in 10–12 week old animals. As serum lipids and triglycerides vary greatly between animals, it is more appropriate to normalize each animal to its baseline value.

We generally defined active compounds *in vivo* as those, which markedly reduced non-HDL-C and TG while elevating HDL-C. Compounds having methylene spacer chain lengths of four and five, cycloalkyl or *gem*-dimethyl substitution, showed the best activity, with no marked difference between diacid and bis-hydroxymethylene analogs.

Symmetrical compounds with two chains of five methylene spacers (**9**, **12** and **28**) were superior to symmetrical compounds with two chains of four (**10**, **11** and **19**) and unsymmetrical compounds with chains of four and five (e.g., **27**) or three and four (e.g., **20**) methylene spacers.

Values for the six (**29**) and seven (**13**, **18**) methylene spacer compounds showed reduction in activity compared to five-methylene spacer compounds (**4**, **6**, **9**, **12**, **28**).

Table 1: Effect of studied compounds on lipid synthesis in primary rat hepatocytes

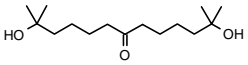
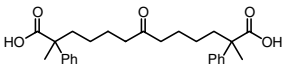
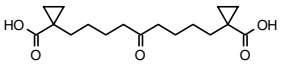
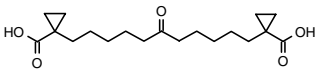
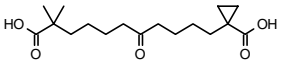
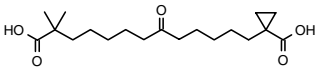
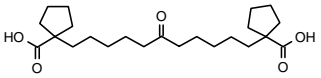
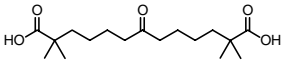
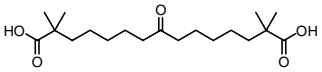
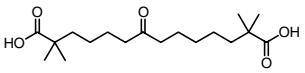
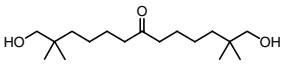
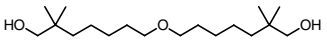
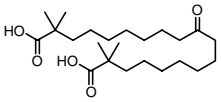
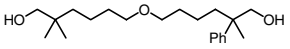
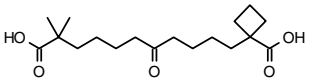
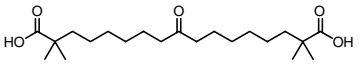
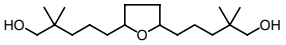
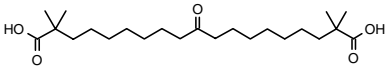
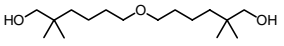
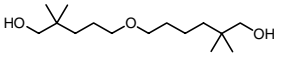
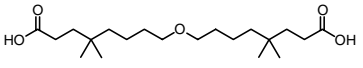
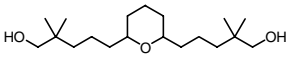
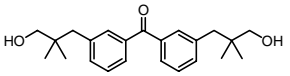
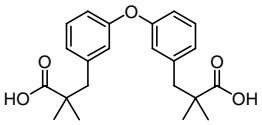
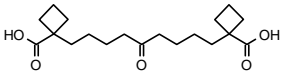
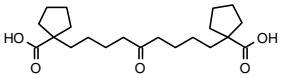
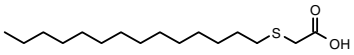
Compd.	Structure	IC_{50} (μM)	95% Confidence interval		r^{2a}
			Lower	Upper	
1		NA[b]			0.32
2		NA[b]			
3		0.5	0.3	6	0.99
4		0.5	0.4	0.7	0.99
5		0.6	0.3	0.9	0.98
6		1	0.7	1.4	0.94
7		2	1.5	2.3	0.99
8		3	2	4	0.93
9		3	2	3	0.99
10		3	3	4	0.96
11		2	0.2	11	0.97
12		4	2	6	0.99
13		5	2	11	0.98
14		17	12	23	0.99
15		6	5	8	0.95
16		9	8	9	1
17		9			0.99
18		14	6	32	0.97
19		11	9	12	0.99
20		17	11	28	0.99
21		22	3	200	0.99
22		39	15	106	0.99
23		42	27	64	0.91

Table 1: (Continued)

Compd.	Structure	IC_{50} (μM)	95% Confidence interval		r^{2a}
			Lower	Upper	
24		53	32	86	0.89
25		121	11	1268	0.89
26		113	7	1794	0.95
3-thia fatty acid		43	28	64	0.99

[a] r^2 is the goodness of fit of the data to the non-linear regression model
[b] N.A., not active, data do not generate an IC_{50} using the non-linear regression model

Table 2: Effect of studied compounds in Female Obese Zucker Rats (Mueller 2004a, b; Bell 2005)

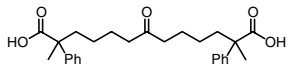
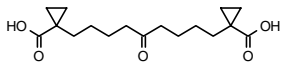
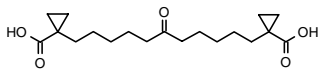
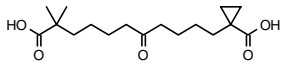
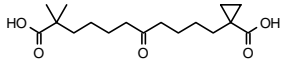
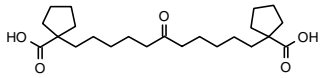
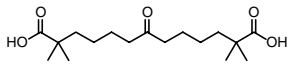
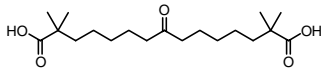
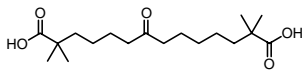
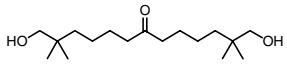
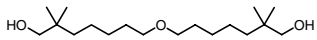
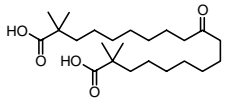
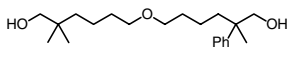
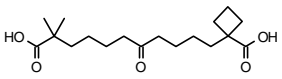
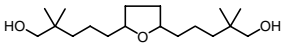
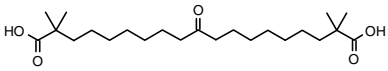
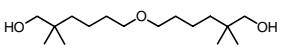
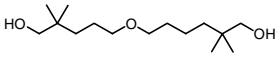
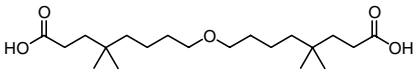
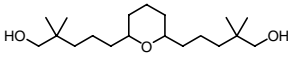
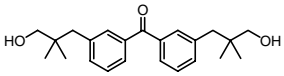
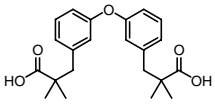
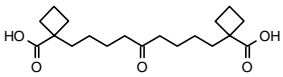
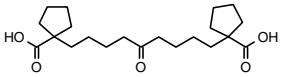
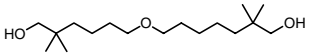
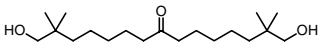
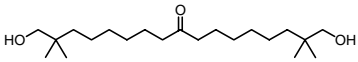
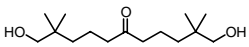
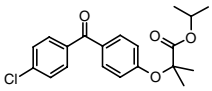
Compd.	Serum Variables (Percent Change from Pre-Treatment)[a]								Structure
	Dose (mg/kg)	No. animals	NonHDL-Cholesterol		HDL-Cholesterol		TG		
			1wk	2wk	1wk	2wk	1wk	2wk	
2	100	3	36	79	43	45	−36	−8	
3	100	3	22	63	180	260	−51	−28	
4	100	4	−92	−83	136	171	−95	−94	
5	100	3	−84	−20	104	248	−93	−64	
6	100	4	−90	−99	43	84	−93	−98	
7	100	4	−54	−32	12	27	−63	−48	
8	100	5	−62	−41	54	78	−75	−69	
9	100	4	−98	−99	72	168	−93	−94	
10	100	5	−62	−41	54	78	−75	−69	
11	100	4	−23	−10	111	126	−54	−29	
12	100	4	−82	−68	99	133	−91	−82	
13	100	3	−81	−45	44	86	−85	−63	
14	100	3	−55	−13	26	52	−61	−45	

Table 2: (Continued)

Compd.	Serum Variables (Percent Change from Pre-Treatment)[a]								Structure
	Dose (mg/kg)	No. animals	NonHDL-Cholesterol		HDL-Cholesterol		TG		
			1wk	2wk	1wk	2wk	1wk	2wk	
15	100	4	4	28	30	60	−54	−51	
17	97	3	11	31	287	140	−49	−13	
18	100	3	−80	−45	44	86	−85	−64	
19	100	4	−38	11	234	366	−77	−71	
20	30	4	44	74	24	40	21	32	
21	100	3	−50	−30	13	39	−80	−73	
22	100	3	−70	−31	75	127	−74	−46	
23	100	4	−14	−5	1	−11	−29	−8	
24	100	4	−59	−21	20	16	−46	−27	
25	100	4	−32	−40	−1	10	−58	−59	
26	100	4	−68	−67	36	40	−67	−70	
27	30	4	−26	17	71	105	−66	−77	
28	100	3	−91	−88	77	135	−92	−92	
29	30	4	−50	−32	85	61	−58	−33	
30	100	3	−22	28	24	2	−31	10	
Reference Agent	100	3	19.8	11.6	−41	−14	6	−8	 Fenofibrate (ref)
Control	0	66	2 (29 ± 19)	−3 (27 ± 10)	−8 (43 ± 19)	−10 (42 ± 20)	0 (1038 ± 49)	−2 (1012 ± 375)	Control[b]

[a] 100% represents a 2-fold increase from pre-treatment value
[b] Lipid parameters are expressed as the group average calculated as percent of pre-bleed for each animal. For the vehicle treatment group (Control) absolute concentration of average serum lipids are also given as mg/dL ± standard deviation in parenthesis

Compounds with a single phenyl substituent to one of the *gem* sites on the quaternary carbon (e.g., **14**) were relatively inactive.

Compounds with cyclopropyl substitutions (**3** and **4**) were more active than the corresponding ones with *gem*-dimethyl substitution (**8** and **9**, respectively).

In some cases, the change in serum variables diminished instead of increasing when 1 week was compared to 2 weeks of treatment (e.g., compounds **10**–**12**). The effect may be due to the well-known cholesterol synthesis compensation mechanism earlier described for statins as operative in rodents (Bisgaier et al. 1997). These effects are known as induction, where enzymes inhibited respond by increasing their synthesis to overcome the block. In addition, drug-metabolizing enzymes are also induced, thereby effectively reducing the amount of active agent. Therefore, agents that are initially active can later be rendered inactive by the body's "induction" response.

The *in vitro* lipid synthesis assay does not necessarily predict the *in vivo* activity because there are multiple absoption, distribution, metabolism and elimination (ADME) factors that affect drug exposures. Additionally, there are potentially multiple MOAs for these types of compounds. However, there is certain reliability with respect to certain structural features (e.g., *gem*-dimethyl substitutions and chains consisting of four or more methylene spacers on both sides of the central carbonyl moiety).

A comparison of activities in the *in vitro* lipid synthesis assay (Table 1) and the Zucker rat model (Table 2) indicated that 14 out of the 16 compounds with an IC_{50} lower or equal to 15 μM in the lipid synthesis assay favorably altered non-HDL-C, HDL-C, and triglycerides in the female obese Zucker fatty rat, except for compounds **15** and **17** that – although they increased HDL-C – did not affect significantly non-HDL-C and triglycerides. Other discordances have been discussed in detail previously (Mueller et al. 2004a, b; Bell et al. 2005).

Clearly, one can appreciate the complexity of the whole animal compared to a cell. It is well known that properties including ADME, as well as enzyme induction will all play a role in the pharmacological effects of a compound. Therefore, it is expected that we would observe difference between the effects of a compound in isolated cells in a test tube versus effects observed in the whole animal.

Overall, we observed a good predictable relation between observations *in vitro* and *in vivo* for reduction of non-HDL-C and showed some, albeit not perfect, predictability between the *in vitro* and *in vivo* tests.

2.4. Conclusion

Fatty acid-like compounds with central ether and ketone functionalities were assessed for their effects on the de novo lipogenesis using radiolabeled acetate incorporation into lipids in primary cultures of rat hepatocytes as well as for their effects on lipid, glycemic and body weight variables in female obese Zucker fatty rats following one and two weeks of oral administration. In both the ether and the ketone series, the most active compounds were found to be symmetrical with four to five CH_2 groups separating the central ether or ketone functionality, respectively, from the *gem* dimethyl, cycloalkyl or methyl/aryl substituents.

Furthermore, biological activity was found to be greatest for cyclopropyl substituted ketone derivatives (Bell et al. 2005). The most promising ether compound **11** ($n = m = 4$, $R^1 = R^2 = CH_3$) was exceptional at elevating HDL-C and also lowered serum TG and non-esterified fatty acids *in vivo*. In the ketone series, symmetrical compounds with four and five CH_2 spacers were the most promising candidates for further development. Ketodiacid **4** ($X = CO$, $n = m = 5$, $R^1 = R^2 =$ cyclopropyl) was identified as an HDL-C elevator and also was found to reduce insulin and glucose.

Our series of compounds have shown excellent preclinical serum lipid regulating properties and HDL-C elevation, and therefore may also have great potential in preventing and treating cardiovascular disorders. Although our SAR studies have been performed on preclinical models for dyslipidemia known to be the best at this moment, we should keep in mind that compounds in this research area may have multiple mechanisms of action and some researchers may re-examine them in other *in vitro* or *in vivo* models. Our approach answers specific questions related to drug design in the metabolic disease area, where QSAR is not predictive because of the uncertainty raised by multiple MOAs.

3. Experimental

Ether compounds were prepared as described in Dasseux and Oniciu (2002a, 2002b) and Mueller et al. (2004a). Syntheses of ketone compounds with *gem*-dimethyl and -(methyl-aryl) substitutions were described in Mueller et al. (2004b). The preparation of cycloalkyl derivatives was described in Bell et al. (2005). Biological measurements were described in Mueller et al. (2004a, 2004b) and Bell et al. (2005): *in vitro* measurement of lipid synthesis in isolated hepatocytes was performed on primary cultures of male Sprague-Dawley rat hepatocytes, while *in vivo* effects on lipid variables were determined in obese female Zucker fatty rats Crl:(Zuc)-faBR.

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