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PERSPECTIVE

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The Lipogenesis Pathway as a Cancer Target

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INTRODUCTION

Lipids constitute some of nature's most abundant molecules, being found in virtually all forms of life. They are derived by condensation reactions involving acetate units and may be divided into three broad categories:

(1) Isoprenoids are also known as terpenoids or prenols. These compounds are constructed from five-carbon units, which themselves are the consequence of earlier steps commencing with acetate. This group encompasses sterols, steroids, secosteroids, bile acids, quinones, and hydroquinones, among others.

(2) Polyketides or acetogenins represent a plethora of poly-β-ketoacid derived secondary metabolites often encountered in macrolide, aromatic, and polycyclic structures.

(3) Fatty acids, the subject of this Perspective, are characterized by the presence of long chains of carbon atoms that impart distinctive hydrophobic character to that portion of whatever molecule they are a component. The chain generally terminates in a carboxyl, aldehyde, or alcohol functionality capable of forming ester, ether, or amide bonds with a wide variety of compounds, including proteins (proteolipids) and complex carbohydrates (saccharolipids, glycolipids, lipopolysaccharides, or lipoglycans). The eicosanoids (prostaglandins and leukotrienes) and sphingolipids (ceramides) also are included among the members of this group. Examples of the diversity of fatty acid derived substances are seen in Figure 1.

The enzymatic processes by which the carbon chains of fatty acids are constructed have been a focus of biochemical research for over half a century, and more continues to be learned as X-ray crystallographic analyses provide increasingly detailed insight into the mechanisms by which these enzymes operate. In recent years there has been considerable interest in the role of these lipogenic enzymes in diabetes and obesity, as well as in the pathogenesis of cancer and as potential therapeutic targets in the search for new antitumor drugs.[1] The latter has stemmed principally from numerous reports linking overexpression of several of the enzymes involved in de novo lipid biosynthesis with various human tumors and by recent advances in the structural biology of these key proteins. These enzymes include acetyl CoA carboxylase, fatty acid synthase, and ATP-citrate lyase, which operate in sequence and represent the primary linkage between carbohydrates and lipids in the cell's metabolic economy. The objectives of this Perspective are to (1) provide an overview of the lipogenic process, primarily as it relates to these three enzymes, (2) examine the relationship of lipogenesis to tumor biology, and (3) outline the opportunities and prospects for targeting the process in the search for new anticancer drugs.

OVERVIEW OF FATTY ACID BIOSYNTHESIS

Long chain fatty acids play a key role in several cellular functions. As triglycerides in adipose tissue, they represent an important energy reserve capable of mobilization to meet the body's energy needs through β-oxidation. They also are essential components of bilayer cell membranes to which they give structural integrity. Also, as membrane-embedded phospholipids and glycolipids, they are capable of recruiting and anchoring proteins involved in signal transduction. In addition, they serve as precursors of important second messengers, such as diacylglycerols, ceramides, eicosanoids, and lysophosphatidic acid. Moreover, lipids are associated with certain specialized functions, for example, the formation of lung surfactant and milk production.

The de novo pathway to long-chain fatty acids can be considered to begin with pyruvate, the end product of anaerobic glycolysis in the cytosol. The overall scheme and the relationships among the key metabolites involved are shown in Figure 2. Pyruvate, following transport into mitochondria, is decarboxylated and converted to acetyl CoA by the pyruvate dehydrogenase complex. Citrate synthase-catalyzed condensation of acetyl CoA with oxaloacetate (OAA) produces citrate, a member of the tricarboxylic acid (Krebs) cycle. Alternatively, citrate can be transported by a translocase from mitochondria to the cytosol, where ATP-citrate lyase (ACLY) catalyzes its conversion back to acetyl CoA and OAA, a step required by the inability of acetyl CoA to cross the mitochondrial membrane.

In the committed step in the pathway to fatty acids, acetyl CoA carboxylase (ACC), a biotin-dependent enzyme found in the endoplasmic reticulum of most eukaryotes, irreversibly carboxylates acetyl CoA to form malonyl CoA. This is followed by the conversion of malonyl CoA and acetyl CoA to a long-chain fatty acid, primarily palmitate, in a remarkable series of steps, all catalyzed by fatty acid synthase (FASN). The overall transformation effected by FASN, the details of which will be examined later, may be represented as follows:

$$\text{acetyl CoA} + 7\,\text{malonyl CoA} + 14\,\text{NADPH} + 14\,\text{H}^+ \rightarrow \text{palmitate} + 7\,\text{CO}_2 + 8\,\text{CoA} + 14\,\text{NADP}^+ + 6\,\text{H}_2\text{O}$$

Elongation by elongases of palmitate (C-16) to stearate (C-18) and higher homologues, known as very long chain fatty acids, occurs in the endoplasmic reticulum. Unsaturation can be introduced into the chain at the C-9 carbon (cis orientation) of both palmitate and stearate by stearoyl CoA desaturase 1 (SCD1). The newly synthesized fatty acids, either per se or as

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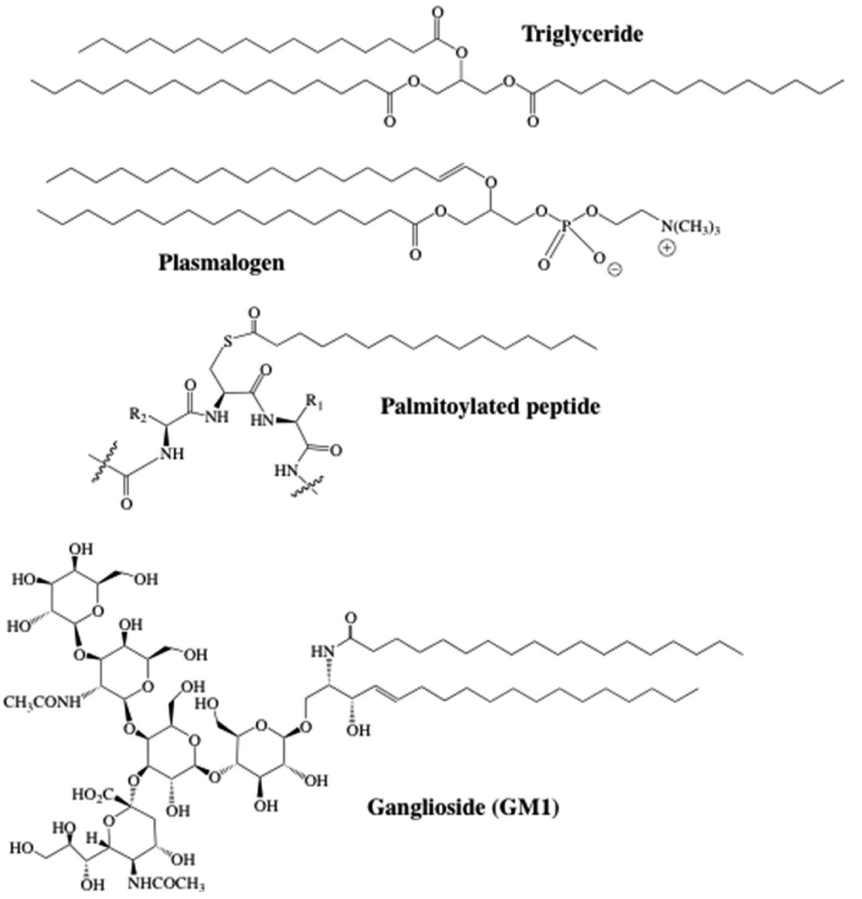

Figure 1. Examples of biologically important fatty acid derivatives.

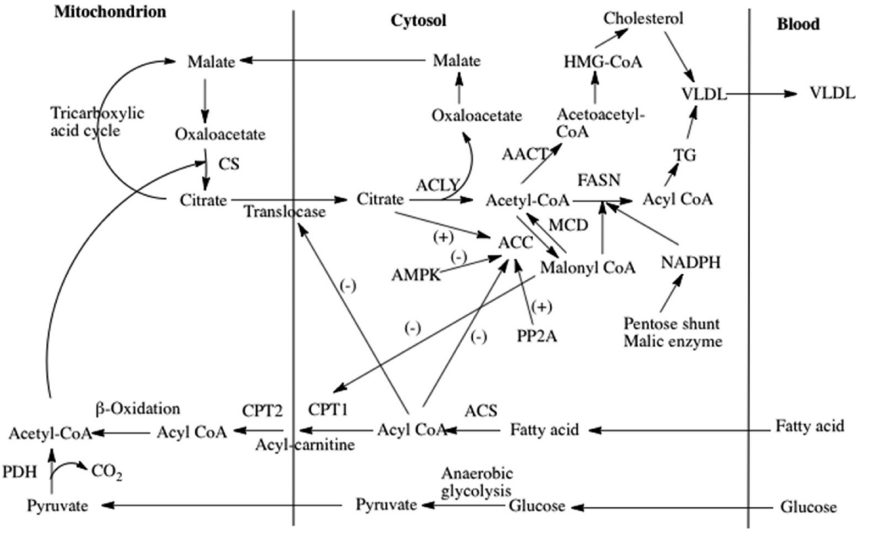

Figure 2. Overview of fatty acid metabolism in the liver and its linkage with carbohydrate metabolism. Reproduced with modifications with permission from *Current Medicinal Chemistry: Immunology, Endocrine & Metabolic Agents* (Groot, P. H. E.; Pearce, N. J.; Gribble, A. D. ATP-citrate lyase: a potential target for hypolipidemic intervention. *Curr. Med. Chem.: Immunol., Endocr. Metab. Agents* **2003**, 3 (3), 211−217).[2] Copyright 2003 Bentham Science Publishers.

their CoA esters, are available for synthesis of triglycerides, sphingolipids, glycolipids, or phospholipids or for transport from the liver as very low density lipoproteins (VLDL).

ACC constitutes the key control enzyme in the fatty acid synthetic pathway.[3] Control is exercised both allosterically and by phosphorylation/dephosphorylation at various serine

residues, as well as at the level of transcription. Phosphorylation by AMP-dependent protein kinase (AMPK) at three serine residues (79, 1200, and 1215) inactivates the enzyme, while protein phosphatase 2A (PP2A) dephosphorylation causes activation. Since AMPK is activated by AMP and inhibited by ATP, this kinase acts as a sensor of the fuel needs of the cell. Thus, when the cell's energy level (ATP) is low, AMPK activity is stimulated and fatty acid synthesis is thereby suppressed.

Hormonal control of ACC activity is affected by insulin, which stimulates its activity through dephosphorylation, while epinephrine and glucagon have the opposite effect. In addition, ACC is feed-forward allosterically regulated by citrate, which stimulates the enzyme's activity. Palmitoyl CoA also plays a key role by inactivating ACC both by blocking the transport of citrate from mitochondria to cytosol and by inhibiting glucose 6-phosphate dehydrogenase, a component of the pentose phosphate shunt, which supplies a portion of the NADPH needed for the reductive steps conducted by FASN. Coenzyme A also has been found to activate ACC, an effect that can be blocked by palmitoyl CoA. Furthermore, malonyl CoA, the product of the ACC step, inhibits carnitine palmitoyltransferase I (CPT-1), thereby blocking entry of fatty acyl CoA esters into the mitochondria, a requisite step in the β oxidation of fatty acids. Malonyl CoA also is known to stimulate insulin secretion from pancreatic β cells.[4]

Additional control of ACC is exercised by BRCA1, whose C-terminal tandem domain (BRCT) directly associates with ACC to block dephosphorylation of Ser-79,[5] and by aldo−keto reductase family 1 member B10 (AKR1B10), which binds to ACC and blocks its degradation by the ubiquitination−proteasome pathway.[6] Thus, *BRCA1* mutations may serve to activate ACC and stimulate fatty acid synthesis while AKR1B10, which is overexpressed in several types of cancer,[7] is a potential target for modulation of fatty acid synthesis in tumor cells.[6] Another mechanism for control of malonyl CoA activity involves its decarboxylation back to acetyl CoA by malonyl CoA decarboxylase (MCD).

Regulation of lipogenesis also occurs at the transcription level primarily by means of carbohydrate response element binding protein (ChERBP)[8] and sterol response element binding protein 1c (SREBP-1c),[9] both members of the basic helix−loop−helix leucine zipper family of transcription factors. The latter stimulates transcription of the genes involved in cholesterol synthesis, as well as the *FASN*, *ACC*, and *ACLY* genes, among others, while the former up-regulates ACC and FASN.[10] ChERBP is activated by glucose[10] and SREBP-1c by insulin through the phosphatidyl 3-inositol kinase (PI3K)-Akt/PKB signal transduction pathway.[11] Thyroid hormone-inducible hepatic protein (also known as SPOT14 or S14), a nuclear protein regulated by SREBP-1c, is another key player in the lipogenic system that controls expression of several enzymes, including FASN.[12] Moreover, transcription of the *FASN* gene is repressed by polyunsaturated fatty acids, sterols, and leptin, all acting to down-regulate SREBP-1c.[13] An excellent review by Sul and Smith[14] provides a detailed discussion and analysis of eukaryotic fatty acid synthesis and its regulation.

LIPOGENESIS AND CANCER

Numerous studies have demonstrated that in adults most tissues obtain fatty acids from the diet via the circulation. Important exceptions to this are the liver and adipose tissue and certain normal proliferative processes, e.g., in the fetal lung for surfactant production, in adults during lactation, in sex-hormone-sensitive cells, and in the cycling endometrium. Consequently, lipogenesis and expression of the involved enzymes generally are low in most adult tissues, including those with normally high proliferative rates such as hematopoietic cells and epithelia of the stomach and small intestine. However, cancer cells are known to synthesize fatty acids at significantly higher rates than normal cells, without regard to extracellular lipid levels. This difference in dependence on lipogenesis between normal and transformed cells thus renders the lipogenic pathway an attractive target for development of antitumor drugs with potentially favorable therapeutic indices.[1]

The association between metabolic phenotype and cancer dates from the 1920s when Otto Warburg reported that cancer cells have a remarkable capacity to consume glucose and generate lactate through aerobic glycolysis (the Warburg effect). The high rate of carbon flux through the glycolytic pathway, from hexokinase to FASN, now is recognized as one of the hallmarks of the malignant phenotype.[15] The reliance of cancer cells on glycolysis, in spite of the presence of high oxygen tension and the inferior ATP-generating ability of glycolysis, to satisfy energy needs is in stark contrast to normal cellular dependence on oxidative phosphorylation for ATP production. For a more in-depth examination of this phenomenon and its causation, the reader is referred to the recent review on this topic by Fulda et al.[16]

It has been known since the early 1950s that rodent cancer cells exhibit a propensity toward elevated lipid synthesis using glucose as the carbon source, even in the presence of an adequate supply of dietary fatty acids. A key step in unraveling the relationship between cancer and lipogenesis was the report of Kuhajda[17] that oncogenic antigen-519 (OA-519), a protein linked to poor prognosis in breast cancer patients, was identical to FASN. Since that initial finding a wide variety of solid human tumors, including cancers of the breast, prostate, colorectum, endometrium, bladder, kidney (nephroblastoma), skin (melanoma), esophagus, tongue, lung, and ovaries, have been found to overexpress FASN, as well as ACLY and ACC.[18] In many cases, the overexpression is found to occur early in tumor progression in the more aggressive tumors associated with unfavorable outcomes.[19,20] To further emphasize the importance of lipid synthesis to cancer cell growth and survival, FASN has been found to comprise as much as 28% of the soluble protein in SKBr-3 cells,[21] a human breast cancer cell line noted for its especially high constitutive expression of FASN, as well as of the human epidermal growth factor receptor (HER2). Furthermore, the key role of lipogenesis in cancer has been demonstrated by numerous studies showing that inhibition of lipogenesis in cancer cells by RNA interference (RNAi)[22] or pharmacological agents[19] leads to suppressed DNA replication, cell cycle arrest, and apoptosis. In addition, FASN overexpression and the resulting overproduction of palmitate have been shown to reduce sensitivity to chemotherapeutic agents while reduction in FASN expression increases drug sensitivity, thus suggesting a possible role for FASN inhibitors as chemosensitizing agents.[23]

The up-regulated lipogenic pathway in tumor cells also may prove useful as a basis for imaging tumors as a possible substitute for ^{18}F-fluoro-2-deoxy-D-glucose (^{18}F-FDG), the standard agent for use in positron emission tomography (PET) in diagnosing many malignancies predicated on the avidity that tumors demonstrate for glucose uptake and metabolism. Since some tumors, e.g., prostate, have low metabolic rates and ^{18}F-FDG may not be ideal for detecting these malignancies, 1-^{11}C-acetate,

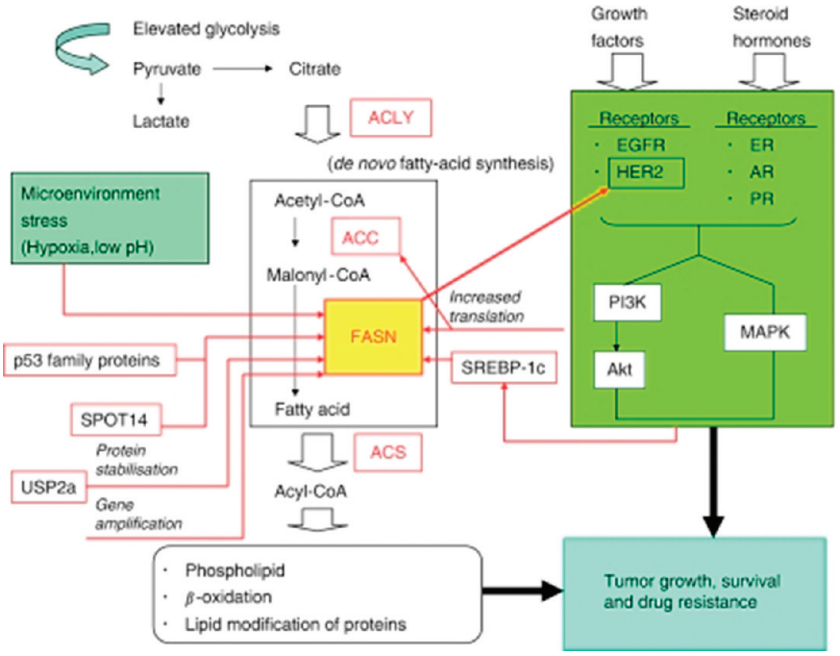

Figure 3. Linkage of growth factor signaling to fatty acid metabolism in cancer cells. Reprinted by permission from Macmillan Publishers Ltd.: *British Journal of Cancer* (http://www.nature.com/bjc/index.html) (Mashima, T.; Seimiya, H.; Tsuruo, T. De novo fatty-acid synthesis and related pathways as molecular targets for cancer therapy. *Br. J. Cancer* **2009**, *100*, 1369−1372),[25] Copyright 2009.

as a lipid precursor, has received recent attention as a potential alternative marker for prostate cancer and its metastases.[24]

The mechanism of FASN overexpression in cancer has been the subject of several studies; however, the total picture remains poorly understood (see Figure 3). In many cases the up-regulation of several enzymes constituting the "lipogenic set"[26] appears to be coordinated.[2,27,28] Involvement of growth factors and their receptors, such as epidermal growth factor (EGF), HER2/neu, and keratinocyte growth factor, has been demonstrated,[29,30] as has the transmission of the cellular signals through the PI3K-PKB/Akt and MAP kinase pathways.[31] For example, it is well-documented that HER2/*neu* overexpression leads to constitutive up-regulation of FASN activity,[29,32] which contributes to tumor cell proliferation, survival, chemoresistance, and metastasis.[33,34] Conversely, blockage of lipogenesis by pharmacologic inhibition of FASN or siRNA silencing of the *FASN* gene in cancer cells turns off the oncogenic action of HER2/*neu*, ultimately leading to apoptotic tumor cell death and prompting speculation of a possible role for FASN inhibitors in therapies of HER2-overexpressing tumors.[35] In addition, induced expression of FASN was found to stimulate cell growth in noncancerous epithelial cells in vitro, resulting in an invasive cancer-like phenotype possessing reduced sensitivity to two tyrosine kinase inhibitors, gefitinib (a HER1 inhibitor) and lapatinib (a HER1/HER2 dual inhibitor). Furthermore, Jin et al.[36] have demonstrated that in breast cancer cells FASN itself is phosphorylated while complexed with HER2, which is hypothesized to result in signaling augmentation and promotion of tumor progression. Oliveras-Ferraros et al.[37] have suggested that circulating FASN levels may have therapeutic implications in determining response to lapatinib in HER2-positive breast cancer patients. Other lipogenic regulatory molecules, including SPOT14 and SREBP-1c, also are amplified in transformed cells,[28] particularly in aggressive tumors. These reports, among others, serve to suggest that a complex cross-talk mechanism involving lipogenic enzymes, their regulators, and surface receptors exists in breast cancer cells.[38]

Bandyopadhyay[39] has demonstrated an inverse relationship in prostate cancer between FASN overexpression and expression of the tumor suppressor gene *PTEN*, whose protein product inactivates PI3K through dephosphorylation. Steroidal sex hormones and their receptors also have been correlated with FASN expression in tumor cells.[40] Moreover, there is evidence that FASN and ACC expression in cancer cells is subject to regulation at the level of translation through mechanisms dependent on Akt, PI3K, and m-TOR.[29] The deubiquitinating enzyme ubiquitin-specific protease 2a (USP2a), which is overexpressed in prostate cancer cells and regulated by androgens, stabilizes FASN by protecting it from degradation by the proteasome. On the other hand, USP2a inactivation decreases FASN levels and enhances apoptosis.[41]

There has been much speculation in the literature concerning the physiological function underlying increased lipogenesis in cancer cells. Among the theories that have emerged to explain this observation is a need to increase the pool of substrates needed for membrane synthesis[19] necessitated by cell division and/or certain membrane-associated lipids, such as phosphatidylinositols, involved in signal transduction pathways critical to sustain cellular proliferation. It also has been hypothesized that increased lipogenesis is needed to compensate for a limited oxygen supply, especially if angiogenesis is insufficient to meet cellular demands. Migita et al.[42] have suggested that in prostate cancer *FASN* itself may be an oncogene whose overexpression protects transformed cells from apoptosis. Accumulation in non-adipose cells of palmitate and related saturated fatty acids, as well as neutral fats, also is well-known to stimulate lipolysis and apoptosis.[43] Cell death in this case may occur through the ceramide pathway via serine palmitoyl transferase-catalyzed condensation of palmitoyl CoA and serine, although a ceramide-independent pathway also may be operative.[44] This phenomenon,

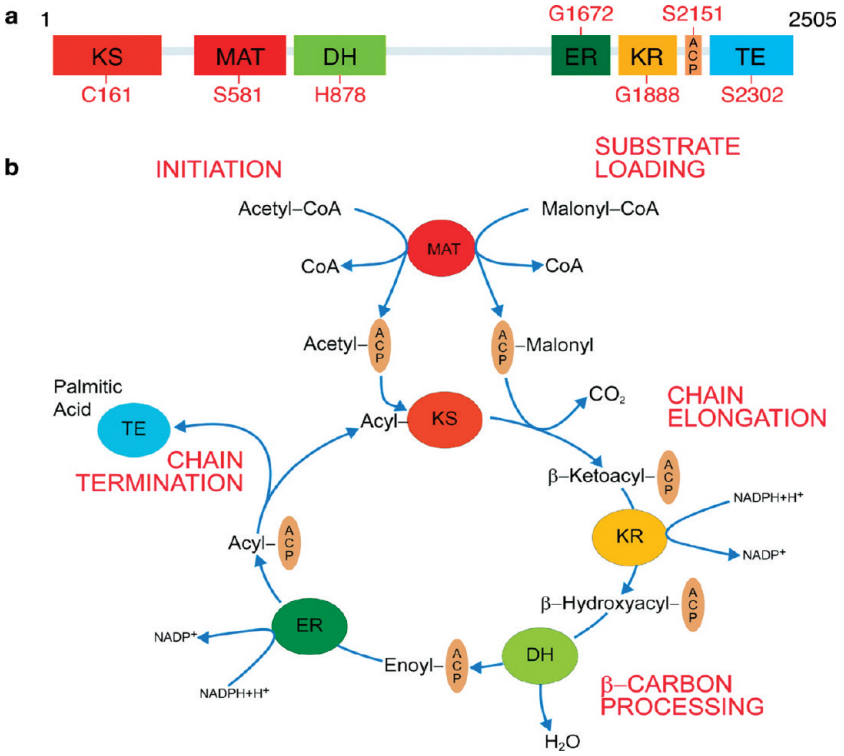

Figure 4. (a) Active sites in the FASN monomer. (b) Cycle of reactions catalyzed by FASN. Reproduced with permission from *ACS Chemical Biology*.[53] Copyright 2006 American Chemical Society.

known as "lipotoxicity", can be blocked by monounsaturated fatty acids[45] and stimulation of peroxisome proliferator-activated receptor γ (PPAR-γ).[46] In a recent review Menendez[30] has suggested that cancer cells avert the toxic effects of palmitate by HER2 stimulation of palmitate incorporation into triglycerides in a PPAR-γ-dependent manner and proposed that HER2-driven tumors may be treated with a combination of lipogenic enzyme inhibitors, PPAR-γ blockers, and possibly dietary unsaturated fatty acids. On the other hand, FASN inhibition recently was reported to enhance the antitumor effects of the PPAR-γ agonist rosiglitazone in prostate cancer cell lines.[47]

FATTY ACID SYNTHASE (FASN)

In mammals, fungi, and lower eukaryotes, the entire reaction sequence from acetyl CoA and malonyl CoA to palmitoyl CoA and palmitate is conducted on a single multifunctional polypeptide chain,[48] sometimes referred to as FASN type I, encoded by a single gene.[49] In contrast, in plants and bacteria, the process is carried out on individual polypeptides and is known as FASN type II fatty acid biosynthesis.[50] In certain bacteria, such as *Mycobacterium tuberculosis*, which makes some unusually long fatty acids, both systems are operative.[51] A third system, which resembles the FASN type II system, has been found in the mitochondria of a number of species, including humans.[52] In animals FASN is a 270 kDa protein containing two identical subunits arranged head-to-tail, a spatial arrangement that enables fatty acid assembly to occur at the interface. Dissociation of the dimer into its component monomers results in loss of FASN activity. In lower eukaryotes the two chains are organized as a heterodimer. Each subunit of the animal enzyme is comprised of an acyl carrier protein (ACP) domain and six different catalytic domains.[54] The structural and functional organization of these domains facilitates the individual reactions carried out in the lengthy iterative process. Starting from the C-terminus, the domains are arranged in the following order (Figure 4a): TE (thioesterase), ACP, KR (β-keto reductase), ER (enoyl reductase), DH (dehydratase), MAT (malonyl/acetyl transferase), and KS (β-ketoacyl synthase); a linker of approximately 800 amino acids separates the DH and ER domains.[55]

The crystal structures of FASN from both fungal and mammalian sources have been determined in a series of elegant studies by Maier.[56] While the crystal structures of five of the domains of the mammalian enzyme now are known, the tethered ACP and TE domains remain unresolved. The homodimer is X-shaped with a condensing (chain-extending) part (KS and MAT domains) and a chain-modifying component (KR, DH, ER, and TE domains), which enclose two reaction chambers (Figure 5).[57] Using single-particle electron microscopy, Brignole et al.[58] determined that individual FASN domains possess sufficient flexibility to permit delivery of substrates to individual catalytic sites within the reaction chambers. Recently, Pappenberger et al.[59] published the crystal structure of the human FASN KS-MAT didomain connected by a linker, pointing out the presence of a small but significant cavity in the KS domain of the human enzyme that is absent in the porcine orthologue. On the basis of their observations, they proposed that it should be possible to generate the isolated human KS domain for use as a tool in the rapid screening of KS inhibitors.

The activity of FASN is initiated when acetyl CoA transfers its acetyl group to the serine-581 OH group in the MAT domain. The acetyl group is then transferred to the SH of the cysteine-161 in the KS domain of one chain of the dimeric enzyme. Meanwhile, the malonyl moiety is first transferred from malonyl CoA

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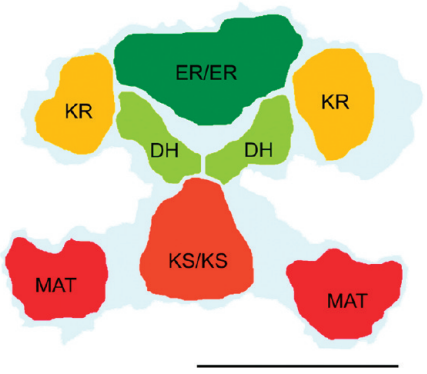


Figure 5. Organization of the FASN active sites overlaid on an outline of the X-ray structure. The ACP and TE domains are not identified but are possibly accounted for by the extra density noted to the right of the KR domain in the monomer seen on the right. The open areas surrounded by the MAT, KS, DH, and KR domains of each monomer are ascribed to the reaction chamber. The scale bar corresponds to 100 Å. Reproduced with permission from *ACS Chemical Biology*.[53] Copyright 2006 American Chemical Society.

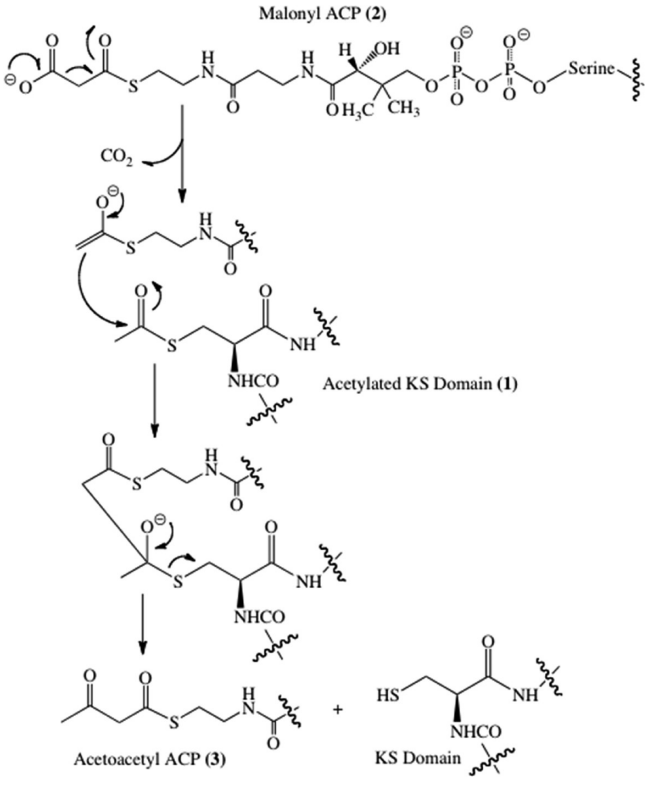


Figure 6. Conversion of malonyl ACP to acetoacetyl ACP.

to the MAT site and then to the cysteamine-SH terminus of the ACP 4′-phosphopantetheine prosthetic group on the opposite chain of the dimer with concomitant loss of CO_2. Juxtapositioning of the acetylated KS domain (**1**) on one chain and the malonyl ACP (**2**) on the other chain sets up a Claisen-type reaction resulting in formation of the acetoacetyl ACP ester (**3**) (Figure 6). The ACP domain through its pantetheine arm, which possesses considerable flexibility, sequentially presents the growing fatty acyl chain to each of the other catalytic

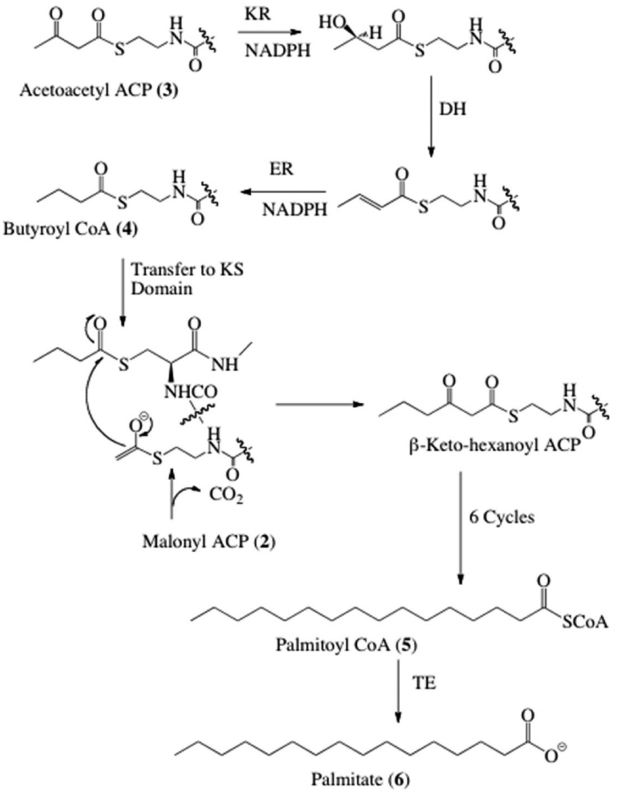
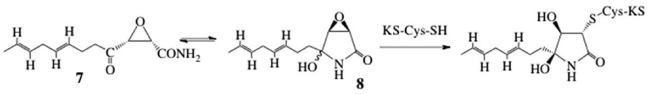


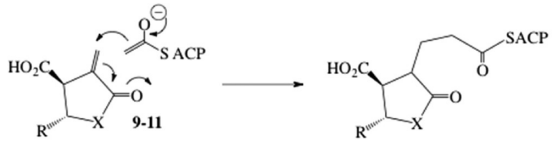
Figure 7. Conversion of acetoacetyl ACP to palmitate.

domains on the opposing chain. At the conclusion of the first series of steps, culminating in butyroyl ACP (**4**), the newly synthesized four-carbon chain is transferred to the KS domain to begin a second round of reactions. The dimeric enzyme has full-site reactivity, i.e., chain formation and extension proceed simultaneously on both chains of the dimer.[60] This process is repeated six times to form palmitoyl CoA (**5**) or in some cases seven times to produce stearoyl CoA, which is cleaved by the catalytic triad of the TE domain to release palmitate (**6**) or stearate (Figure 7). This triad, consisting of serine 2308, histidine 2481, and aspartate 2338,[61] is a conserved arrangement from insects to mammals.

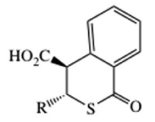
As noted earlier, blockade of FASN activity inhibits the growth and proliferation of cancer cells. Although the exact mechanism by which this occurs is unknown, a number of possibilities exist based on experimental evidence.[34,54] For example, it is known that at least some cancer cells appear to acquire an obligatory dependence on de novo fatty acid synthesis to provide membrane building blocks, since exogenously added palmitate or oleate is unable to reverse the effects of FASN inhibition.[33] Reduced palmitate levels in the cell may also be reflected in diminished palmitoylation of key intracellular signaling proteins, such as H-ras and N-ras.[62] Palmitoylation has been shown to be necessary for anchoring of ras proteins to the cell membrane in order to realize the full transforming activity of the oncogene.[63] Another possible mechanism involves toxic accumulation of malonyl CoA, which triggers up-regulation and nuclear accumulation of the transcription factor PEA3, which in turn binds to the *HER2* promoter.[64] Occupancy at this site by PEA3 blocks access of the TATA binding protein to the TATA box, thus repressing the *HER2* promoter and ultimately transcription of the *HER2* oncogene.[65] Furthermore, malonyl CoA also up-regulates ceramide production with consequent induction of the proapoptotic



7[72]; IC_{50} = 21.86 μM (FASN from A375 malignant melanoma cells)



9; X = O, R = *n*-C_8H_{17}; IC_{50} = 15.53 μM (rat liver FASN)[73], 32.43 μM (FASN from A375 malignant melanoma cells)[72]
10[73]; X = S, R = *n*-C_8H_{17}; IC_{50} = 2.56 μM (rat liver FASN)
11[73]; X = S, R = *n*-$C_{11}H_{23}$; IC_{50} = 0.79 μM (rat liver FASN)



12[74]; R = *n*-C_8H_{17}; IC_{50} = 12.32 μM (rat liver FASN)
13[74]; R = *n*-C_9H_{19}; IC_{50} = 8.29 μM (rat liver FASN)

Figure 8. Compounds **7** and **9** and related fatty acid synthase inhibitors.

genes *BNIP3*, *TRAIL*, and *DAPK2*.[22] Another contributing factor may relate to the p53 status of the tumor cell, since loss of p53 function renders tumor cells more sensitive to FASN inhibition.[66] Additional possible explanations relate to the disruption of the earlier described extensive cross-talk involving up-regulation of FASN and signal transduction pathways.

Cerulenin (**7**), an antifungal antibiotic, is an established FASN inhibitor that in protic solvents exists in equilibrium with its diastereomeric hydroxylactam (**8**).[67,68] Compound **7** has been found to react irreversibly with FASN by forming a covalent bond with a cysteine sulfhydryl group in the KS domain of type I FASN.[69,70] The crystal structure of **7** complexed with FASN from *S. cerevisiae* identified C1305 as the site of formation of the covalent linkage, formed by opening of the epoxide ring at the C-2 position of the chain, thus inhibiting initial transfer of the acyl moiety onto the catalytic cysteine.[70] This results in two significant conformational changes involving F1646 and M1251 that block fatty acid synthesis. Funabashi[69] has shown that reaction of *E. coli* FASN involves formation of the C-2 opened epoxide in its hydroxylactam form.

Compound **7** (Figure 8) has demonstrated significant in vitro cytotoxicity toward tumor cells, triggering apoptosis, delaying disease progression, and resulting in increased survival of human cancer xenografts. However, the reactive epoxide ring makes **7** susceptible to other nonspecific ring-opening reactions that limit any potential clinical application.[34]

trans-4-Carboxy-5-*n*-octyl-3-methylenebutyrolactone (C75, **9**) lacks the epoxide present in **7** and consequently is more stable and specific in terms of its chemical reactivity compared to **7**.[71] The design of **9** was based on the mechanism of the thioester Claisen condensation in which the enolate anion, derived from decarboxylation of **2**, attacks the *S*-acyl group on the KS domain (see Figure 7). In the case of **9** the enolate, or possibly the catalytic cysteine SH group,[59] presumably attacks the electrophilic α-methylene lactone in Michael fashion. Consistent with this mechanism, kinetic studies revealed that **9** is a slow-binding irreversible inhibitor of mammalian FASN.[71] Furthermore, reduction of the exocyclic double bond of **9** provides a derivative (C273) lacking in cytotoxicity or FASN inhibitory activity.[75] However, molecular docking studies with **9** and related compounds indicate that the inhibitory action may be due to noncovalent bonding interaction with the KS domain.[73] Using rat liver FASN, Rendina and Cheng[76] provide evidence that **9** inactivates the ER and TE domains, as well as the KS domain. In addition, **9** has been found to bind to the TE domain of human FASN in an orientation similar to that of palmitate with the hydrocarbon chain projecting into a hydrophobic pocket and the hydrophilic lactone interacting with the catalytic triad.[61]

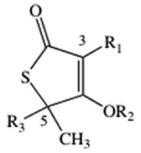
Compound **9** exhibits in vitro cytotoxicity against a number of human tumor cell lines[35] and is active in vivo against human cancer cell xenografts.[32] Apoptosis induced by **7** and **9** appears related to malonyl CoA accumulation.[66,75,77] On the other hand, **9** causes anorexia and loss of body weight, effects not particularly beneficial in an anticancer drug candidate, regardless of potential for a favorable therapeutic index. The effects on body weight and appetite are attributed to inhibition of neuropeptide Y in the hypothalamus and to stimulation of carnitine palmitoyltransferase-1 (CPT-1) and consequent activation of mitochondrial fatty acid oxidation, all of which are additional effects associated with increased cellular levels of malonyl CoA.[78] Compound **9** also has shown promise as a potential chemopreventive agent for breast tumors.[32]

The thiolactone analogue (**10**) of **9** demonstrated enhanced FASN inhibitory activity compared to **9**. Lengthening the side chain at position 2 of **10** out to C_{11} (**11**) improved the FASN inhibitory activity even further to below 1 μM.[73] Recently, isothiochroman-4-carboxylic acid derivatives **12** and **13** were found to be more effective FASN inhibitors and have higher therapeutic indices than **9**.[74]

(5*R*)-Thiolactomycin (Table 1, **14a**) is a broad-spectrum thiotetronic acid-based antibiotic that inhibits type II FASN with reportedly very little affect on the type I enzyme,[79] although this selectivity has been challenged in a more recent study in which **14a** showed considerable activity against human FASN from breast cancer cells.[80] Compound **14a** acts as an isosteric malonyl thioester analogue to reversibly inhibit bacterial 3-ketoacyl-ACP synthase (KAS).[81] McFadden et al.[80] reported that modifications to **14a** at positions 3 (compounds **15a** and **17**) and 5 (compounds **16**−**18**) resulted in potent inhibition of type I FASN and effective antitumor activity without causing substantial weight loss. Other workers have shown that loss of the 3-methyl, coupled with inversion of the configuration at C-5 from (*R*) to (*S*) (compounds **14b** and **15b**) also enhances type I FASN inhibitory activity.[82] However, the 5-epimerization may be less important in this regard than loss of the 3-methyl group, since FASN I inhibitor C247 (**16**) also displays favorable antitumor effects without causing weight loss.[32] C93 (or FAS93, structure not disclosed), an orally effective FASN I inhibitor apparently based on **14a**, has been the subject of considerable recent interest because of its anticancer activity and negligible ability to produce anorexia and weight loss.[23]

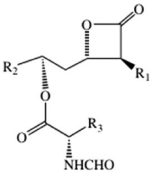
Orlistat (Figure 9, **19**) or (−)-tetrahydrolipstatin, a polyketide-like β-lactone currently approved by the FDA for obesity management, is an inhibitor of gastric and pancreatic lipases in the gastrointestinal tract. Compound **19** works by acylating the active site serine in the TE domain by opening of the β-lactone ring,[83] assisted by the aspartate and histidine residues in the catalytic triad, as has been noted for binding of **9** at the same site.[61] The crystal structure of the human FASN TE domain bound to **19** captured the inhibitor both as a stable acylated enzyme intermediate and as the hydrolyzed product with the

Table 1. Compound 14a and Analogues



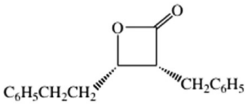
compd	R_1	R_2	R_3	inhibition of fatty acid synthesis, IC_{50} (μM)	cytotoxicity, IC_{50} (μM)[a]	% weight loss[b]
14a: (5*R*)[79,80,82]	CH_3	H	C_5H_7[c]	1.90[d]		
				>951[e]		
				>380[f]		
				100[g]	>380	2.6[h]
14b: (5*S*)[82]	CH_3	H	C_5H_7[c]	208[f]		
15a: (5*R*)[80]	H	H	C_5H_7[c]	42.8[g]	>407	7.8
15b: (5*S*)[82]	H	H	C_5H_7[c]	96.8[f]		
16: (5*R*)[80]	H	H	*n*-C_8H_{17}	16.5[g]	72.6	2.0
17: (5*R*)[80]	$CH(OH)CH_3$	H	*n*-C_8H_{17}	11.5[g]	73.3	0.2
18: (5*R*)[80]	H	CO_2CH_3	*n*-C_8H_{17}	6.99[g]	71.9	6.1

[a] Cytotoxicity to cultured MCF-7 human breast cancer cells.[80] [b] Weight loss in Balb/C mice 1 week following a single 60 mg/kg ip dose.[80] [c] (*E*)-$CH{=}C(CH_3)CH{=}CH_2$ [d] Inhibition of type II *E. coli* FabB.[79] [e] Inhibition of type I FASN from rat liver.[79] [f] Inhibition of type I FASN from human HepG2.[82] [g] Inhibition of type I FASN from human ZR-75-1 breast cancer cells.[80] [h] Using **14a**/**14b** racemic mixture.[80]

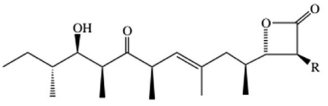


Compound	R_1	R_2	R_3	IC_{50} (μM)[a]
19[85]	*n*-C_6H_{13}	$CH_2CH(CH_3)_2$	*n*-$C_{11}H_{23}$	1.35
20[85]	C_2H_5	H	*n*-$C_{11}H_{23}$	0.23
21[85]	*n*-C_6H_{13}	$CH_2CH(CH_3)_2$	$CH_2CH_2CH{=}CH_2$	0.28
22[85]	*n*-C_6H_{13}	$CH(CH_3)_2$	*n*-C_5H_{11}	0.30
23[85]	*n*-C_8H_{17}	$CH_2CH(CH_3)_2$	*n*-C_9H_{19}	3.00

[a] Inhibition of purified TE domain of FASN as measured by fluorogenic assay



24[86]
K_i = 2.5 μM; **19** K_i = 0.28 μM
(recombinant FASN TE domain)



25[87]: R = CH_3
26[87]: R = C_2H_5

Figure 9. β-Lactone FASN inhibitors.

palmitic chain projecting into the hydrophobic substrate binding pocket and the hexyl chain packed against the histidine of the catalytic triad.[84] Although **19** has demonstrated antitumor activity in tumor cell lines, as well as in mouse models,[87] the prospect of developing **19** as an anticancer drug is limited by its poor solubility and low oral bioavailability. In a systematic SAR study of 28 β-lactones Richardson et al.[85] identified two derivatives (**20** and **21**) and the related natural product valilactone (**22**) as inhibiting the FASN TE domain at concentrations 1 order of magnitude lower than that of **19**. An isomer (**23**) of **19**, in which the lengths of the two carbon chains are transposed, was about 3 times less potent than **19** as a FASN TE inhibitor. Purohit et al.[86] have prepared a series of β-lactone ketene dimers and studied their inhibitory effects on a recombinant form of the TE domain of FASN. The best inhibitor proved to be the *cis*-3-benzyl-4-phenylethyl derivative (**24**) whose K_i compared favorably with that of **19**. The naturally occurring β-lactones ebelactones A (**25**) and B (**26**) inhibit FASN TE but are less selective than **19** as inhibitors of the enzyme in tumor cells.[87] Panclicin D, another β-lactone closely related to **19**, is more potent than **19** as a lipase inhibitor; however, its activity against FASN has not been reported. Activity-based protein profiling using alkyne-bearing derivatives of **19** and "click"-based azides enabled identification of nine protein targets, including FASN, although discerning the significance of these additional targets in relation to the pharmacological effects of **19** requires further study.[88]

Epidemiological studies have suggested that consumption of green tea may have chemopreventive effects in humans.[89] The precise mechanism by which cancer risk may be reduced has been the subject of much speculation, frequently focused on the antioxidant properties of its polyphenolic constituents, although inhibition of FASN has gained favor in recent years as a possible explanation for this effect. The most abundant polyphenols in this popular beverage are flavanols of the catechin type, the most plentiful being (−)-epigallocatechin gallate (EGCG, **27**), which has been reported to induce apoptosis and cell cycle arrest in human cancer cells as a result of FASN inhibition without increasing CPT-1 activity.[90,91] FASN inhibition by **27** occurs at the enzyme's KR domain and is biphasic with a rapid, reversible binding phase followed by slow, irreversible inactivation, while the ER domain and its NADPH binding site are not affected. Inhibition is competitive with NADPH, the galloyl group probably acting on the KR NADPH binding site.[92] Moreover, in an SAR study of green tea catechins, Wang et al.[93] concluded that the galloyl ester attached to ring C, in which the carbonyl group is highly electron deficient and subject to attack by a nucleophilic group in the KR domain, was required for optimum FASN inhibition by catechins. However, not all catechin derivatives may

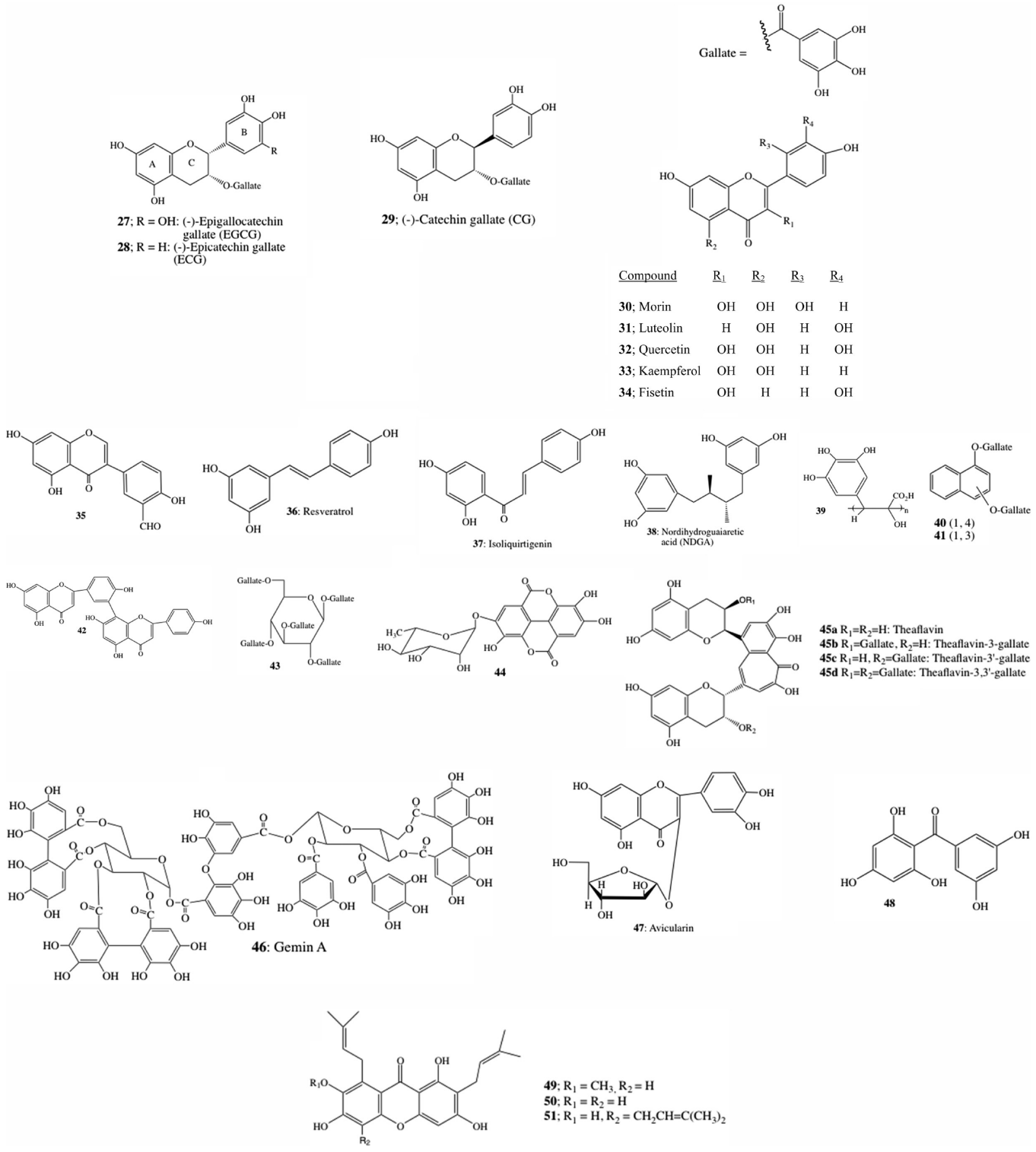


Figure 10. Polyphenols with FASN inhibitory properties.

act at the FASN KR site as was shown by Zhang et al.[94] for (−)-catechin gallate (**29**), which appears to interact at FASN's AT domain. Studies[68,94−99] on a wide array of natural polyphenols (see Figure 10 and Table 2) has led to the proposal of a more general model shared by many polyphenolic FASN inhibitors, including the catechin derivatives. The model features (1) two aromatic rings, each bearing OH groups, (2) a linker measuring approximately 0.67 nm between the two rings that maintains them in a coplanar (or nearly so) relationship, and (3) if one of the aromatic rings is part of an ester, the carbonyl be sufficiently electrophilic to permit facile attack from a site within the KR domain. Interestingly, Zhang et al.[100] reported that highly potent FASN inhibitors with IC_{50} values in the single-digit micromolar range and possessing significant activity against

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Table 2. FASN Inhibitory and Cytotoxic Properties of Polyphenols

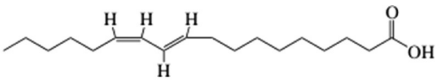
	FASN inhibition IC_{50} (μM)		
compd	overall	KR[a]	cytotoxicity, IC_{50} (μM)
27[90,92]	52[b]	100[b]	150[c]
28[93]	42[b]	68[b]	
29[94]	3.4[d]	30.1[d]	
30[98]	2.3[d]	26[d]	
31[68,98,99]	31.4;[f] 2.52[d]		71.2[e]
32[68,98,99]	39.7;[f] 4.29[d]		30.9[e]
33[68,98,99]	174;[f] 10.4[d]		>400[e]
34[98,99]	18.8[d]		>400[e]
35[68]	7.7[f]		
36[95]	8.5[d]	120[d]	
37[95]	8.8[d]	141[d]	
38[95]	9.3[d]	427[d]	
39[100]	2.59[b,g]	17.6[b,g]	
40[101]	*h*		29[c]
41[101]	*i*		21[c]
42[68]	13.0[f]		
43[68]	2.98[f]		
44[68]	17.0[f]		
45[96,97]	1.77[b,j]	5.00[b,j]	
46[102]	0.21[d]		40.4[k]
47[97]	6.15[b]		
48[103]	8.59[b]		
49[103]	5.54[b]		
50[103]	1.24[b]		
51[103]	3.30[b]		

[a] Reduction of FASN ketoreductase (KR) activity. [b] Chicken liver FASN. [c] SKBr-3 cells. [d] Duck liver FASN. [e] HepG2 cells. [f] *Saccharomyces cerevisiae* FASN. [g] Based on proposed monomeric product (MW = 212.16) in ref 100. [h] Inhibited FASN activity by 69% at the IC_{50} cytotoxic level. [i] Inhibited FASN activity by 90% at the IC_{50} cytotoxic level. [j] Mixture of **45a**, **45b**, **45c**, and **45d**. [k] BGC-823 gastric cancer cells.

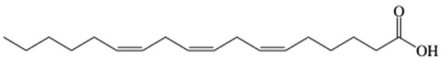
human breast cancer cells could be obtained by heating catechins, both gallated and ungallated, in acid. The structures of the reaction products were deduced from spectral analysis to be 2-hydroxypropenoic acid oligomers; e.g., the product derived from **27** was proposed to have structure **39**.

Puig et al.[101] synthesized a series of simple naphthalenediol bis-galloyl esters for study as FASN inhibitors with anticancer properties. Two of the compounds (**40** and **41**) demonstrated substantial activity against FASN and remarkable cytotoxicity while not inducing CPT-1 stimulation or weight loss. These may serve as prototypes for future FASN inhibitor development. Liu et al.[102] reported the isolation of several ellagitannins from *Geum japonicum* that demonstrated reversible fast-binding inhibition against duck liver FASN at or near submicromolar levels, the most potent being **46**. Substantial FASN inhibitory activity also has been found in some benzophenone (**48**) and xanthone (**49**−**51**) constituents of *Garcinia mangostana*.[103]

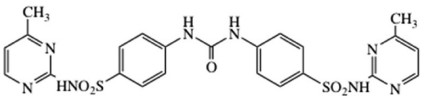
One of the more controversial areas in connection with dietary approaches to cancer treatment and prevention concerns the effects of polyunsaturated fatty acids (PUFAs). With regard to FASN in particular two such compounds have attracted attention.



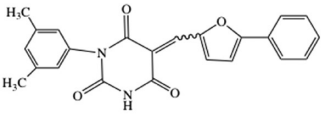
52[104]: IC_{50} = 100 μM (FASN from SKBr-3 cells)



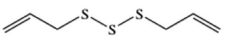
53[105]: FASN from SKBr-3 cells inhibited 41% at 6.25 μg/ml (22.4 μM) and 78% at 50 μg/ml (179 μM)



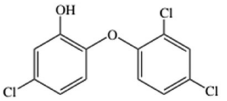
54[107]: IC_{50} = 15.8 nM (rhFASN)



55[108]: K_i = 0.38 μM (rhFASN TE); cytotoxicity: IC_{50} = 3.47 μM (MDA-435 cells)



56[109]: IC_{50} = 8.37 μM (Chicken liver FASN)



57[110]: IC_{50} = 10-20 μM (goose uropygial gland FASN; value dependent on assay conditions)

Figure 11. Additional FASN inhibitors.

10*E*,12*Z*-Conjugated linoleic acid (CLA, **52**, Figure 11), the major conjugated linoleic acid isomer found in several products derived from ruminant animals, has been found to both down-regulate and inhibit FASN and to suppress the growth of human tumor cell lines.[104] γ-Linolenic acid (GLA, **53**), an essential ω-6 polyunsaturated fatty acid, has shown selective toxicity to human breast cancer cells, an effect generally attributed to **53**-induced peroxidation and free radical formation.[105] However, it has been proposed that **53** itself is oxidized to its 9,10- and/or 11,12-epoxide, which act as direct inhibitors of FASN.[106] Dihomo-γ-linolenic acid, arachidonic acid, and eicosapentaenoic acid, as well as other PUFAs that show tumor cell toxicity, may behave similarly.

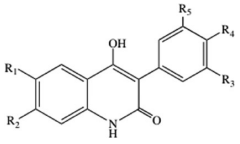
The symmetrical ureide GSK837149A (**54**) was identified as a potent type I FASN inhibitor in the course of a high-throughput screening program. This bisulfonamide reversibly inhibited FASN by binding to the KR site with a K_i in the 25−35 μM region; however, **54** and its analogues displayed very poor cell permeability.[107] In another high-throughput screening approach Richardson and Smith[108] identified a pharmacophore based on pyrimidine-2,4,6-trione and having the ability to competitively inhibit the thioesterase domain of FASN. These compounds, the most active of which was **55**, killed FASN-dependent breast cancer cells (MDA-MB-435) at concentrations 2- to 5-fold lower than those required to kill nonlipogenic MCF-10A breast cancer cells. Diallyltrisulfide (DATS, allitridin, **56**), a major constituent of *Allium* vegetables, such as garlic and onions, long suspected of having antiproliferative properties, was found to be a potent inhibitor of FASN.[109] Analysis indicated that **56** inactivated the enzyme by irreversibly reacting with the essential sulfhydryl groups on the ACP and KS domains. Triclosan (**57**), a broad spectrum antibacterial widely used in a variety of personal care products,

Table 3. Inhibition of FASN and FASN TE by Peptides Isolated from Soybean β-Conglycinin[111]

peptide	inhibition IC_{50} (μM) FASN[a]	FASN TE[b]
KNPQLR (**58**)	80	ND[c]
EITPEKNPQLR (**59**)	27	10
RKQEEDEDEEQQRE (**60**)	16	11
9	80	59

[a] Chicken liver. [b] Recombinant human. [c] Not determined.

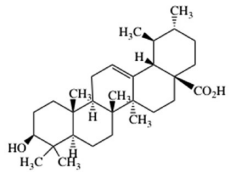
Table 4. FASN Inhibitory Activity of 3-Aryl-4-hydroxyquinolin-2(1*H*)-ones[112]



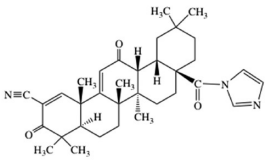
compd	R_1	R_2	R_3	R_4	R_5	FASN inhibition IC_{50} (nM)[a]	FASN inhibition IC_{50} (nM)[b]
61	NO_2	Cl	H	H	H	73	207
62	NO_2	F	H	H	H	50	
63	NO_2	Cl	Cl	H	Cl	134	
64	NO_2	Cl	H	C_6H_5	H	122	
65	NO_2	Cl	H	$OCH_2C_6H_5$	H	86	
66	CN	Cl	H	H	H		136
67	CN	Cl	C_6H_5	H	H		52
68	CN	Cl	C_6H_4-4-F	H	H		54
69	CN	Cl	C_6H_3-3,4-di-F	H	H		68
70	CN	Cl	2-naphthyl	H	H		127
71	CN	Cl	1-benzothien-3-yl	H	H		101
72	CN	Cl	C_6H_4-2-OCH_3	H	H		19

[a] Rat FASN. [b] Human FASN.

not only inhibits the type II FASN ER domain of bacteria but also the avian and mammalian type I ER domain and has been shown to suppress carcinogenesis in rats. It also is effective in cell cultures against SKBr-3 and MCF-7 breast cancer cell lines. It has been postulated that inhibition of the reduction step and the resulting increase in the concentration of the enoyl thioester at the active site renders this moiety subject to Michael addition by proximate nucleophiles, thus enhancing the overall FASN inhibitory activity of **57**.[110] Recently, Martinez-Villaluenga[111] reported the isolation of three FASN-inhibiting peptides (**58**−**60**) from soybean β-conglycinin, two of which had FASN inhibitory properties in the 10 μM range when assayed against recombinant human FASN TE domain (Table 3). Docking studies implied that these peptides blocked the TE active site through interactions with the catalytic triad, the interface cavity, and the hydrophobic groove. The most potent inhibitors of human FASN reported to date were contained in a series of 3-aryl-4-hydroxyquinolin-2(1*H*)-ones (**61**−**72**) studied by Rivkin et al.,[112] many of which were active below 100 nM (Table 4). The design of these compounds was based on the assumption that the enol



73[113]: IC_{50} Chicken liver FASN: 13.1 μM (overall reaction) and 59.3 μM (KR activity)



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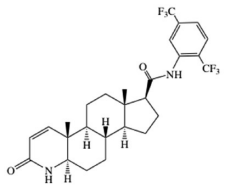

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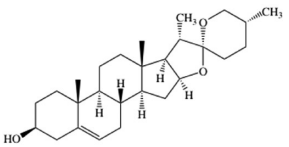

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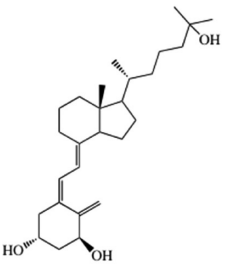

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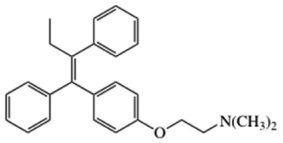

78[118]

Figure 12. Steroidal and antiestrogenic FASN inhibitors.

functionality would mimic the enol intermediate formed in the enzymatic condensation of acetyl CoA and malonyl CoA at the KS domain; however, the site of action of these compounds was not reported.

A number of steroid-based molecules, both natural and synthetic, also have demonstrated FASN inhibitory properties. For example, ursolic acid (**73**, Figure 12), a pentacyclic triterpenoid acid that is widely distributed in plants and a component of several traditional Chinese herbs purported to have anticancer properties, has been found to inhibit FASN.[113] Inhibition initially is reversible at the AMT domain, followed by irreversible inactivation of the enzyme. The synthetic triterpenoid 2-cyano-3,12-dioxooleana-1,9-dien-28-oic imidazole (CDDO-Im, **74**) recently has been reported to down-regulate both FASN and S14 in human liposarcoma cells, a finding that led to the suggestion that a combination of this oleanic acid derivative with a FASN inhibitor, such as **7**, may have synergistic effects in treating tumors.[114] The 5α-reductase 1 and 2 inhibitor dutasteride (**75**) reduces both FASN expression and activity in LNCaP prostate cancer cells in vitro. This effect is associated with reduction in the levels of SREBP1 and its downstream effector SCAP and consequent expression of proapoptotic genes *BNIP3*, *TNFRSF10B*, and *DR5*, leading to cell death.[115] Diosgenin (**76**), a steroidal sapogenin, has been found to block FASN expression in HER2 overexpressing breast cancer cells. The inhibition was accompanied by reduced phosphorylation of Akt and mTOR and up-regulation of JNK phosphorylation, all effects known to be involved in FASN regulation.[116] Several studies have substantiated the association between vitamin D and analogues and reduced cancer risk.[119] Cell cycle arrest at the G_0/G_1 interface through induction of the cyclin-dependent kinase inhibitors p21

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and p27 has been proposed as one mechanism underlying this effect. However, another possible mechanism has been suggested involving up-regulation in prostate cancer cells by 1α,25-dihydroxy vitamin D_3 (calcitriol, **77**) of long-chain fatty acid CoA ligase 3 (FACL3), which preferentially converts myristic acid, eicosapentaenoic acid (EPA), and arachidonic acid to their respective CoA esters. These esters, in turn, down-regulate FASN expression by feedback inhibition.[117] In addition, the anti-estrogen tamoxifen (**78**) has been reported to down-regulate FASN expression and activity, leading to malonyl CoA-induced inhibition of mitochondrial β-oxidation, a major contributing factor to the development of hepatic steatosis, non-alcoholic steatohepatitis, and cirrhosis associated with long-term use of **78**.[118]

■ ACETYL COA CARBOXYLASE

In the committed step in the pathway to fatty acids, acetyl CoA carboxylase (ACC), a biotin-dependent enzyme found in the endoplasmic reticulum of most eukaryotes and possessing two catalytic activities [biotin carboxylase (BC) and carboxyltransferase (CT)], irreversibly carboxylates acetyl CoA to form malonyl CoA.

The enzyme exists in two different isoforms referred to as ACC1 (ACCA or ACCα) and ACC2 (ACCB or ACCβ), each encoded by separate genes and carrying out the conversion of acetyl CoA to malonyl CoA but with distinctly different roles in the cell. The malonyl CoA produced by ACC1 is used by FASN in lipogenesis, this 262 kDa isoform being found predominantly in liver, adipose tissue, and the lactating mammary gland. On the other hand, ACC2 (282 kDa) is confined to the mitochondrial outer membrane in tissues such as skeletal muscle and heart

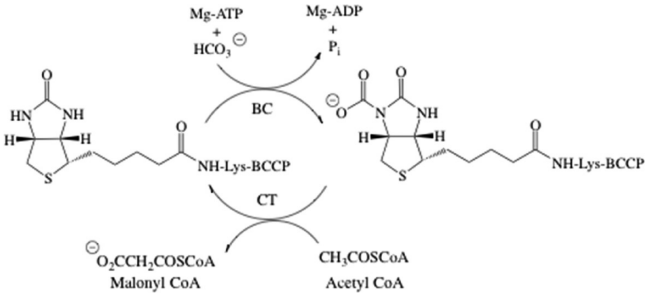

Figure 13. Conversion of acetyl CoA to malonyl CoA by ACC: BC, biotin carboxylase; CT, carboxyltransferase; BCCP, biotin carboxyl carrier protein.

where β-oxidation plays a major role; in this case the malonyl CoA acts as an allosteric inhibitor of CPT-1 to regulate the process by which fatty acids are oxidized for energy. Because of their differing functions, the malonyl CoA molecules produced by each of the isoforms do not mix but rather are compartmentalized into two independent pools. The isoforms share approximately 75% sequence identity, one of the principal differences being in the additional 140 N-terminal amino acids on ACC2, which is a major factor contributing to its subcellular localization.[120] There also is significant homology among mammalian ACCs; e.g., rat and human ACC1 share 97% sequence identity. Methodology for expressing and isolating recombinant versions of both human isoforms has been described.[121] FASN knockout[122] and ACC1 (but not ACC2) knockout[123] have been found to be embryonically lethal in mutant mice, indicating the essentiality of fatty acid synthesis to in utero development.

The carboxylation of acetyl CoA by ACC is a stepwise process that in eukaryotes utilizes three different domains of a single dimeric multifunctional protein: the BC domain that transfers CO_2 from bicarbonate to biotin in the presence of ATP and Mg^{2+}, yielding carboxybiotin; the CT domain which transfers the carboxyl group from carboxybiotin to acetyl CoA, resulting in malonyl CoA; the biotin carboxyl carrier protein (BCCP) domain to which biotin is covalently attached through a lysine residue and which provides the "swinging arm" that moves between the CT and BC domains (Figure 13).[124] In prokaryotes the steps are performed by three different polypeptides contained in a multimeric complex; the BC and BCCP peptides are homodimeric, while CT is an $\alpha_2\beta_2$ heterotetramer. Although there is considerable sequence homology between the BC domains of bacteria and eukaryotes, CT sequence conservation is much weaker. However, the human and yeast CT domains share 50% overall sequence identity and about 90% identity in the active site region, making the yeast CT domain a suitable surrogate for the study of inhibitor interactions.[125] Several eukaryotic ACC crystal structures have been published (see Table 5).

Because it plays a key role in energy metabolism in general and in fatty acid synthesis in particular, there has been much interest in developing agents to target ACC in the treatment of obesity, diabetes, and the metabolic syndrome,[125−127] as well as cancer.[120] ACC also is a target for herbicide development; compounds such as the cyclohexanediones sethoxydim (**79**) and tepraloxydim (**80**) and the aryloxyphenoxypropionates diclofop (**81**) and haloxyfop (**82**) are reversible inhibitors of grass plastid ACC that block the CT domain (Figure 14). Recent

Table 5. Published Eukaryotic ACC Crystal Structures[a]

source and ref	domain	resoln (Å)	isoform	note
yeast[126]	CT	NS	ACC1	complex with **104** and **106**−**108**
yeast[130]	CT	2.3	NS	complex with **80**
human[131]	CT	3.2	ACC2	complex with **104**
yeast[132]	CT	2.8	NS	complex with **104**
yeast[133]	CT	2.5, 2.8	NS	free enzyme (2.5 Å) and complex with **81** (2.5 Å) and **82** (2.8 Å)
yeast[134]	BC	1.8	NS	free enzyme and complex with **115**
yeast[135]	CT	2.7	NS	free enzyme and complex with CoASH
human[136]	outside BC and CT domains	3.2	ACC1 (residues 1258−1271, phosphorylated at Ser 1263)	complex with human BRCA1 BRCT domains

[a] NS = not stated.

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years also have seen ACC as a potential target in developing new antimicrobial agents. Of particular note in this regard, Mochalkin et al.[128] employed a combination of virtual screening and fragment-based drug discovery approaches to identify a number of structural leads as potential bacterial BC inhibitors. ACC is overexpressed in several types of cancer, including breast,[6,20,29,129] liver,[9] prostate,[27] and liposarcomas.[114] There does not appear to be a differential overexpression of either isoform in cancer cells, although the levels of both ACC isozymes were measured in only two of these reports[20,129] and both were found to be elevated. RNAi silencing of ACC1 produces an effect similar to that observed with silencing of the FASN gene, namely, growth inhibition and apoptosis induction.[29]

While nonisozyme selective ACC inhibitors would appear advantageous from the standpoint of diabetes and obesity, the development of ACC1-selective inhibitors, which would block fatty acid synthesis without stimulating fatty acid oxidation and causing weight loss, would appear to be a desirable objective from the cancer perspective, particularly if combined with a FASN inhibitor. In one of the few direct comparisons available in the literature, Corbett[126] demonstrated that in a series of spirochromanones several compounds, although active below 100 nM, failed to show any selectivity for rat ACC1 over human ACC2 (Tables 6 and 7). A related group of spirochromanones exhibited IC_{50} values vs ACC in the low nanomolar range, but no attempt at differentiating between the isozymes was reported (Table 8).[137]

Haque et al.[138] prepared a series of biphenyl- and 3-phenylpyridine-based inhibitors of ACC, making direct comparisons of inhibitory potency using both recombinant ACC1 and ACC2 from humans (Table 9). Some of these compounds were active against one or both in the low nanomolar range, and three (**104**−**106**) showed good to excellent selectivity for the ACC1 isozyme. The stereochemistry at the chiral center may have a substantial impact on potency, as demonstrated by data for the racemate of 3-phenylpyridine derivative **101** and its individual enantiomers **102** and **103**.

CP-640186 (**104**, Figure 15), which has been shown to interact at the CT domain,[131] was developed by Pfizer as a nonspecific ACC inhibitor for potential use against obesity and diabetes.[139] This scaffold was used recently to synthesize and test a series of 4-piperidinylpiperazines, one of which (**105**) showed modest selectivity against the ACC2 isozyme.[140]

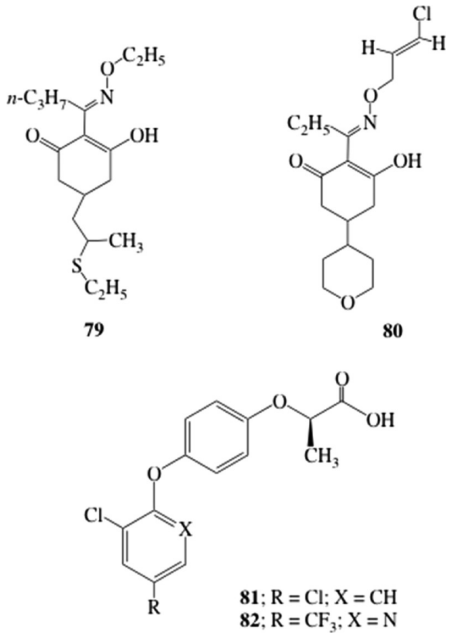
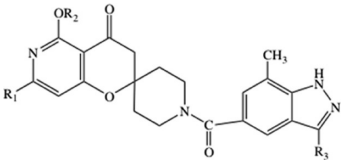


Figure 14. Herbicidal ACC inhibitors.

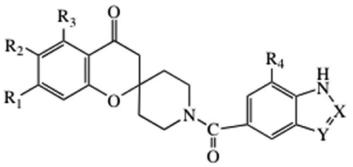
Table 7. Inhibition of ACC Isoforms by 6-Azaspirochromanones[126]



				IC_{50} (nM)		
compd	R_1	R_2	R_3	rACC1[a]	hACC2[b]	ACC1 selectivity[c]
87	H	*i*-Pr	H	30	4.5	0.15
88	CH_3	CH_3	H	34	48	1.41
89	H	*i*-Bu	H	23	98	4.26
90	H	*i*-Pr	CH_3	12	20	1.67

[a] Rat ACC1. [b] Human ACC2. [c] IC_{50}(human ACC2)/IC_{50}(rat ACC1).

Table 6. Inhibition of ACC Isoforms by Spirochromanones[126]



							IC_{50} (nM)		
compd	R_1	R_2	R_3	R_4	X	Y	rACC1[a]	hACC2[b]	ACC1 selectivity[c]
83	CH_3	CH_3	H	CH_3	N	CH	22	48	2.18
84	H	H	OCH_3	H	CC_6H_5	N	16	16	1.00
85	H	2-methyl-4-oxazolyl	H	CH_3	N	CH	19	30	1.58
86	H	2-methyl-5-oxazolyl	H	CH_3	N	CH	17	39	2.29

[a] Rat ACC1. [b] Human ACC2. [c] IC_{50}(human ACC2)/IC_{50}(rat ACC1).

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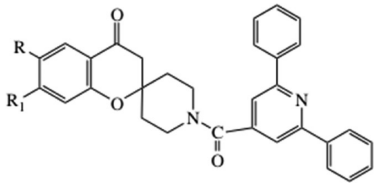
Using high-throughput screening, researchers at Abbott Labs identified a lead compound, designated A-80040 (**109**), with good selectivity for the recombinant human ACC2 isozyme.[141] Follow-up SAR studies produced some derivatives with greater than 1000-fold ACC2 selectivity and/or ACC2 inhibitory potencies in the single-digit nanomolar range (Table 10).[141,142]

Marjanovic et al.[143] have developed an interesting high throughput screen using yeast-gene-replacement strains to identify new ACC inhibitors, including inhibitors of both human isozymes. The screen, which measures yeast growth instead of enzyme activity, correctly identified a number of compounds known to target ACC. As proof of principle, the screen was used to uncover **114** (CD-017-0191), which inhibited human ACC2 while having no inhibitory effect on ACC1 (Figure 16). This technique thus offers the possibility of identifying potent selective inhibitors of the ACC1 isozyme, as well as possible antitumor agents. A group at Bristol-Myers Squibb has developed a single-step homogeneous ACC/FASN coupled assay suitable for high-throughput screening. The method uses [^{3}H]acetyl CoA and detection of the tritiated palmitate product.[144]

Soraphen A (**115**), a naturally occurring macrolide, is an antifungal agent that owes its activity to its ability to inhibit both isoforms of eukaryotic ACC by binding to the BC domain dimer interface and disrupting its oligomerization.[134,145] Beckers[146] has shown **115** to cause apoptosis in prostate cancer cells by blocking fatty acid synthesis and stimulating β-oxidation, thus demonstrating that cancer cells are capable of requiring ACC for their proliferation and survival. Premalignant BPH-1 cells did not undergo apoptosis under the same conditions. Olsen[114] has shown that **115** reduced the growth of two liposarcoma cell lines but had no effect on normal fibroblasts; the antiproliferative action could be countered by palmitate.

5-(Tetradecyloxy)-2-furoic acid (TOFA,**116**), which is converted in vivo to its coenzyme A ester, an allosteric inhibitor of ACC1, has been found to induce apoptosis in lung and colon cancer cells.[147] The mechanism of the inhibitory effect on ACC1 is similar to that of the normal regulatory role played by long

Table 8. Inhibition of ACC by Spirochromanones[137]



compd	R	R_1	ACC inhibition IC_{50} (nM)[a]
91	$NHCO(CH_2)_2CH_3$	H	6
92	morpholin-1-yl	CH_3	2
93	morpholin-1-yl	H	6
94	H	morpholin-1-yl	26
95	H	*N*-methylpiperazin-1-yl	27
96	H	piperidin-1-yl	36
97	2-oxopyrrolidin-1-yl	H	14

[a] Rat skeletal muscle ACC.

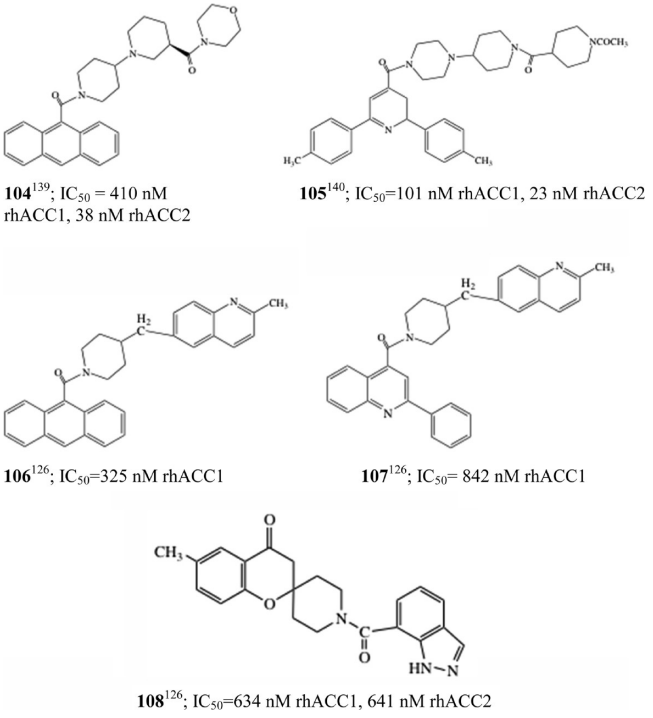
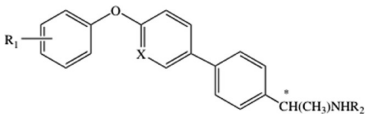


Figure 15. Pfizer ACC inhibitors based on the **104** scaffold.

Table 9. Inhibition of ACC by Phenoxybiphenyl and Phenyoxy-3-phenylpyridine Derivatives[138]



compd	R_1	R_2	X	IC_{50} (nM) rhACC1	IC_{50} (nM) rhACC2	ACC1 selectivity[a]
98	4-benzyl	$COCH_3$	CH	39	945	24.2
99	4-$(CH_2)_2OCH_3$	$COCH_3$	CH	89	>30000	>337.1
100	3-$O(CH_2)_2CH_3$	pyrazol-4-yl	CH	36	609	16.9
101	4-$O(CH_2)_2CH_3$	$COCH_3$	N (*R*, *S*)	110	18	0.16
102	4-$O(CH_2)_2CH_3$	$COCH_3$	N (*S*)	35	8	0.23
103	4-$O(CH_2)_2CH_3$	$COCH_3$	N (*R*)	>30000	777	<0.026

[a] IC_{50}(rhACC2)/IC_{50}(rhACC1).

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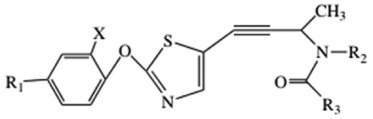
Table 10. Inhibition of ACCby 4-(Thiazol-5-yl)but-3-yn-2-amine Derivatives[141,142]



					IC$_{50}$ (μM)		
compd	R_1	R_2	R_3	X	rhACC1	rhACC2	ACC2 selectivity[a]
109	OC_6H_5	OH	NH_2	H	1.0	0.075	13.3
110	OCH_2-cyclopropyl	H	CH_3	Cl	>30	0.010	>3000
111	O-*i*-Bu	H	CH_3	CH_3	>30	0.009	>3333
112	O-*i*-Pr	H	CH_3	H	>30	0.019	>1579
113	O-*n*-Pr	H	CH_3	H	0.14	0.004	35

[a] IC$_{50}$(rhACC1)/IC$_{50}$(rhACC2).

chain acyl CoA esters. In contrast, Pizer et al.[77] reported that **116** was not cytotoxic to human breast cancer cells, although malonyl CoA production was reduced by 60% and the reduction in fatty acid levels was comparable to that seen with FASN inhibitor **7** or **9**. This, together with the finding that simultaneous exposure of the cells to both **116** and a FASN inhibitor led to reduced apoptosis, suggests that the buildup of malonyl CoA plays the major role in the induction of cytotoxicity.

Several other ACC inhibitors, many of which also require initial in vivo conversion to their CoA esters, have been developed as potential hypolipidemic agents, although their antitumor properties have yet to be reported. These include **117**[148] (MEDICA 16), which is incapable of undergoing β-oxidation, **118**[149] (ESP-55016-CoA), and **119**[150] (S-2E-CoA); the last two also have been shown to inhibit HMG CoA reductase.

Levert and Waldrop[151] prepared the bisubstrate analogue **120** by linking biotin benzyl ester to CoA. This analogue competitively inhibited *E. coli* CT with respect to malonyl CoA (K_i = 23 μM) and had an affinity for the enzyme that was 350-fold greater than that of biotin. The chloroacetylated biotin derivative **121** also was found to inhibit mammalian ACC in 3T3-L1 cells, indicating that it is capable of crossing the cell membrane and being converted to the CoA derivative. Sugimoto et al.[152] identified the benzofuranyl α-pyrone **122** (TEI-B00422) as a highly active competitive inhibitor of ACC in HepG2, a human hepatoma cell line.

■ ATP-CITRATE LYASE (ACLY)

ATP-citrate lyase (ACLY, EC 4.1.3.8), originally known as the "citrate cleavage enzyme", is a tetrameric enzyme belonging to the acyl-CoA synthetase (nucleoside diphosphate-forming) superfamily, whose members also include succinyl CoA synthetase (SCS), acetyl CoA synthetase, and malate thiokinase. The human enzyme was cloned by Elshourbagy et al.[153] who found that the enzyme's four apparently identical subunits each contain 1105 amino acids having a molecular mass of 121 419. Two isoforms of ACLY have been mapped to locus 17q21.2 in the human genome, one having 1101 residues (isoform 1) and the other 1091 residues (isoform 2). The overall reaction catalyzed by ACLY is shown in Figure 17A.

ACLY is one of four enzymes for which citrate is a substrate, the others being bacterial citrate lyase (EC 4.1.3.6) and citrate synthase

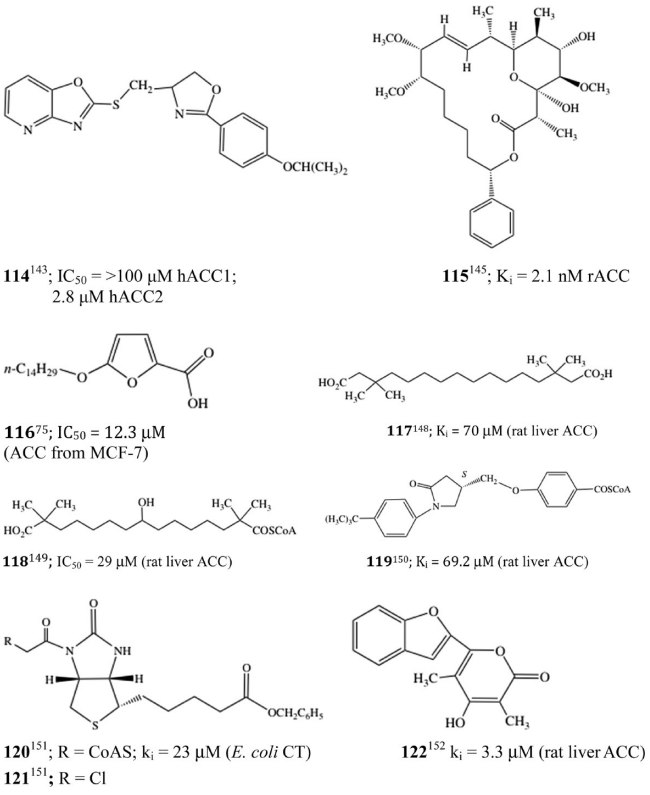


Figure 16. Additional ACC inhibitors.

(EC 4.1.3.7), both of which, like ACLY, catalyze the retro-aldol-type cleavage of citrate to oxaloacetate and either acetate or acetyl CoA, and aconitase (EC 4.2.1.3), which catalyzes citrate's reversible dehydration/hydration to isocitrate in the Krebs cycle.

The accepted mechanism for ACLY's action is that reported in 1991 by Wells et al.[154] Initial autophosphorylation of ACLY by ATP-Mg^{2+} occurs at histidine 764[155] in the human enzyme.[153] In addition to autophosphorylation, the histidine residue at the catalytic site also can be phosphorylated by nucleoside diphosphate kinase and dephosphorylated by protein histidine phosphatase; however, the regulatory significance of this remains unclear.[156] Transfer of the phosphate from histidine to the pro-S carboxylate of citrate ensues. The resulting citrate phosphate anhydride (**123**), while noncovalently bound to the enzyme, is attacked by CoASH to produce the tightly bound *S*-CoA ester (**124**), which is subject to attack by an active site base initiating the retro-Claisen cleavage to oxaloacetate and acetyl CoA (Figure 17B).

All members of the acyl-CoA synthetase (nucleoside diphosphate-forming) superfamily comprise five domains whose numbering is derived from the sequential order as found in the two subunits (α and β) of *E. coli* SCS, the first member of the superfamily whose structure was determined by X-ray crystallography.[157] Domains 1 and 2, found in the *E. coli* SCS α-subunit, contain the CoA binding site and the phosphorylated histidine residue, respectively. The *E. coli* SCS β-subunit is made up of three domains: domain 3 assumes an ATP-grasp fold; domain 4 is the site to which the nucleotide binds; and domain 5 works with domain 2 through their respective amino termini to provide the "power helices" to which the phosphorylated histidine residue is bound.[157] In the human counterpart the analogous domains

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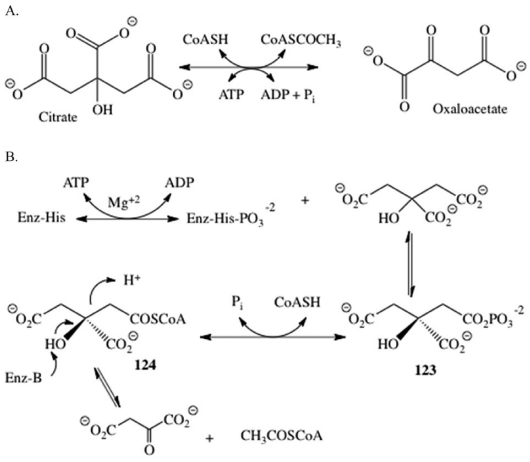


Figure 17. (A) Overall reaction catalyzed by ACLY. (B) Mechanism of the ACLY-catalyzed reaction.[154]

occur in the order 3, 4, 5, 1, and 2 in moving from the N- to the C-terminus of each chain of the tetramer. Recently, the crystal structure of a chymotrypsin-truncated human ACLY, containing all five domains and bound to citrate, has been determined by Sun et al.,[158] providing a clear picture of the citrate-binding site, situated in a loop formed by residues Ser 343 and Thr 348 within domain 5.

Normally, ACLY expression is highest in liver and adipose tissue and lowest in brain, small intestine, and muscle.[159] Using image-enhanced green fluorescent protein-tagged ACLY, Wellen[160] detected the enzyme in the nucleus, as well as in the cytosol, in two different mammalian cell lines. Their data further indicated that ACLY links growth factor-induced uptake and metabolism of glucose to the regulation of histone acetylation and consequent regulation of genes involved in glucose metabolism, including the glucose transporter Glut4, and the enzymes hexokinase 2, phosphofructokinase 1, and lactate dehydrogenase.

In addition to the aforementioned catalytic histidine residue, regulatory phosphorylation of ACLY occurs at Ser 454 (catalyzed by cAMP-dependent protein kinase and promoted by insulin and glucagon) and at Thr 446 and Ser 450 (both catalyzed by glycogen synthase kinase 3). Phosphorylation increases the enzyme's activity 6-fold. It is not clear, however, if ACLY expression is under the control of SREBP-1c, as it is for FASN and ACC.[161]

Because of its upstream position relative to both cholesterol and fatty acid synthesis, most of the focus on this enzyme has concerned its role in the development of obesity and hyperlipidemia.[2,162] However, there are a number of indications that ACLY may be a suitable target for anticancer therapy as well. Expression of ACLY is significantly up-regulated in tumor cells, a finding first reported by Szutowicz et al.[163] in human breast carcinoma cells. Migita et al.[164] reported that ACLY overexpression in non-small-cell lung cancer cells correlated with stage, differentiation grade, and prognosis and that ACLY knockdown resulted in arrest of tumor cell growth both in vivo and in vitro. Likewise, Hatzivassiliou et al.[165] demonstrated that RNAi or pharmacological inhibition of ACLY reduced in vivo tumor growth and induced differentiation in A549 lung adenocarcinoma cells. The antitumor effect was shown to be the result of inhibition of cell cycle progression rather than via apoptosis, leading Knowles et al.[166] to conclude that reduced de novo palmitate synthesis is insufficient to induce tumor cell apoptosis. In addition, ACLY knockdown was found to impair tumorigenesis mediated by Akt, an activator of ACLY through phosphorylation, in a cultured murine prolymphocytic cell line (FL5.12).[167] Yahagi et al.[9] noted a marked elevation in the expression of ACLY, as well as of FASN and ACC in human hepatocellular carcinoma samples from patients. Elevated ACLY expression also has been noted in human bladder cancer.[168] Moreover, prostate cancer cells were characterized by Halliday et al.[169] as having increased ACLY activity, although no direct measurements were done on the enzyme. This conclusion was based on the low levels of citrate secreted from prostate cancer cells compared to their normal counterparts, possibly because of high levels of cytosolic ACLY.[170] Recent work by Beckner et al.[171] indicates that ACLY plays a key role in the invasiveness of glioma cells and that the enzyme may be a suitable target for prevention of the process.

The mechanism linking ACLY activation to cellular transformation is not known. One piece of evidence in the puzzle is a report[167] that ACLY serves as a substrate for Akt/PKB, which is known to suppress apoptosis by phosphorylating a number of proapoptotic proteins and to regulate gene expression at both transcription and translation levels.[172]

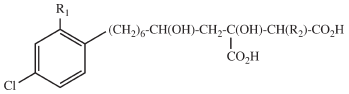
Several inhibitors of ACLY have been studied for their ability to block fatty acid and/or cholesterol biosynthesis, while little emphasis has been placed on their potential role as antitumor drugs. The discussion that follows reviews each of the known ACLY inhibitors along with their pharmacological and/or biochemical effects.

Several citrate analogues (Table 11 and Figure 18) were prepared and studied at laboratories of SmithKline Beecham (now Glaxo SmithKline) in the 1990s as ACLY inhibitors.[2] These included the (3*S*)- and (3*R*)-2,2-difluorocitrates **125** and **126**, both of which demonstrated very good activity against rat liver ACLY, although the absolute configurations of the isolated enantiomers were not determined.[173] A number of (3*R**,5*S**)-ω-substituted-3-carboxy-3,5-dihydroxyalkanoic acid derivatives (**130**−**135**), some of which were active ACLY inhibitors at submicromolar concentrations, also were reported.[174] This same group likewise conceived a series of sulfoximine and β-lactam derivatives of citric acid as potential ACLY inhibitors based on analogy with glutamine synthetase whose mechanism of action, like that of ACLY, involves formation of an intermediate γ-carboxylate phosphate anhydride that is displaced by a nucleophile. Only one of the prepared compounds (**136**) exhibited activity, albeit very weak, against rat liver ACLY.[175] Also prepared was a series of potential active-site directed and mechanism-based ACLY inhibitors containing electrophilic or latent electrophilic groups, although none of the compounds proved to be especially active with the best activity being found in the reversibly binding epoxy citrate derivative (**137**).[176]

A series of benzenesulfonamides, prepared by Li et al.[177] at Bristol-Myers Squibb, were found to be highly effective inhibitors of rhACLY in the low micromolar range. The most active compound in the group was **138** (Figure 19), although it demonstrated no cytotoxicity up to 50 μM. Compound **117**, discussed earlier as an inhibitor of ACC, also exhibits modest activity against ACLY, K_i = 16 μM with respect to citrate and K_i = 3 μM with respect to CoA.[178]

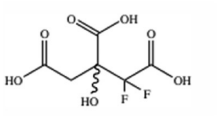
In addition to these synthetic compounds, some natural products also have been found to inhibit ACLY. Ki et al.[179] discovered that radicicol (**139**), a naturally occurring antifungal macrolide, noncompetitively inhibits rat liver ACLY. Compound **139** has

Table 11. Inhibition of ACLY by 2-Substituted 1,4-Butanedioic Acid Derivatives[174]

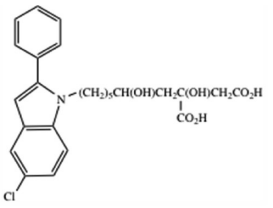


compd	R_1	R_2	stereochemistry	K_i (μM)
130 (SB-201076)	Cl	H	(±)-3*R**,5*S**	1.0,[a] 1.0[b]
131	Cl	OH	(±)-2*R**,3*R**, 5*S**	0.62[b]
132	Cl	OH	(±)-2*R**,3*S**, 5*R**	0.55[b]
133	3,4-dimethylpyrrol-1-yl	H	(±)-3*R**,5*S**	0.3[b]
134	3,4-dimethylpyrrol-1-yl	H	(+)-3*R**,5*S**	0.58[b]
135	3,4-dimethylpyrrol-1-yl	H	(−)-3*R**,5*S**	0.34[b]

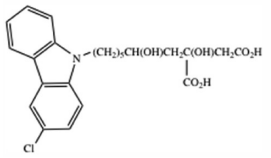
[a] Rat liver ACLY. [b] Recombinant human ACLY.



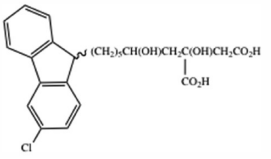
125 (+)[173]: K_i = 0.7 µM (Rat liver ACLY)
126 (-)[173]: K_i = 15 µM (Rat liver ACLY)



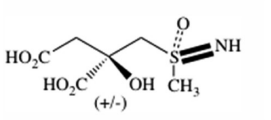
127 (+/-) (3*R**, 5*S**)[174]: K_i = 0.37 µM (rhACLY)



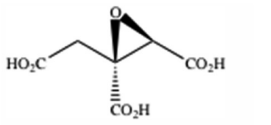
128 (+/-) (3*R**, 5*S**)[174]: K_i = 0.71 µM (rhACLY)



129 (+/-) (3*R**, 5*S**)[174]: K_i = 0.22 µM (rhACLY)

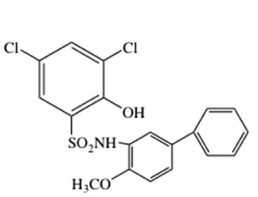


136 (+/-)[175]: K_i = 250 µM (rat liver ACLY)

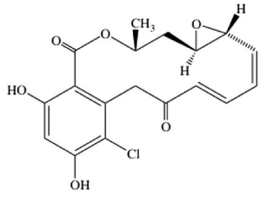


137 (-)[176]: K_i = 18 µM (rat liver ACLY)

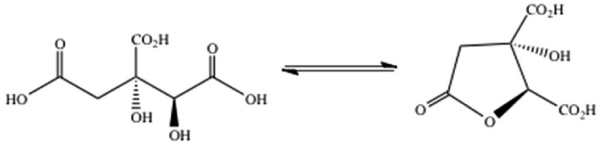
Figure 18. ACLY inhibitors studied by SmithKline Beecham.



138[177]: IC_{50} = 0.13 µM (rhACLY)

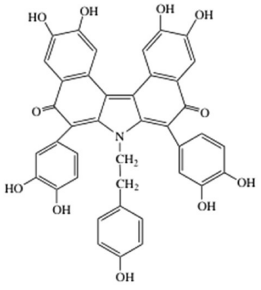


139[179]: K_i = 13 µM for citrate and 7 µM for ATP (rat liver ACLY)

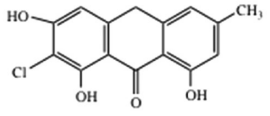


140 (-): K_i = 8 µM (rat liver ACLY)[180] K_i = 0.15 µM (rat liver ACLY)[181]

141 (-)[180]: K_i = 50-100 µM (rat liver ACLY)



142[182]: IC_{50} = 7 µM (rat liver ACLY)



143[183]: IC_{50} = 283 nM (rat liver ACLY)

Figure 19. Additional ACLY inhibitors.

been much more widely studied for its ability to bind to and inhibit heat shock protein 90 (Hsp90) (IC_{50} = 20 nM),[184] its apparent antitumor target, although its antiproliferative capacity has been demonstrated only in vitro. Metabolic conversion of **139** to derivatives lacking affinity for Hsp90 probably accounts for the lack of in vivo antitumor activity.[185] On the other hand, some radicicol analogues, such as radanamycin, a chimera of **139** and geldanamycin,[186] another well-known Hsp90 inhibitor, demonstrate in vivo antitumor activity.

Of the four possible stereoisomers of 2-hydroxycitrate, only the (2*S*,3*S*) isomer (**140**), isolated from the fruit rinds of *Garcinia* species and often referred to in the literature as (−)-hydroxycitrate or HCA, is active as an inhibitor of ACLY.[187] In solution, **140** is found as a mixture with its γ-lactone (**141**), which is much less active as an ACLY inhibitor than is **140**.[180] Schwartz et al.[188] found that a combination of the pyruvate dehydrogenase cofactor α-lipoic acid (8 μM) and calcium hydroxycitrate (300 μM) was effective in initiating complete cell death in murine tumor cell models with no demonstrable toxicity when given to healthy mice.

Another SmithKline Beecham finding, the alkaloid purpurone (**142**), was extracted from a marine sponge of the *Iotrochota* genus and found to inhibit ACLY in a dose-dependent manner, although it showed no cytotoxicity to HepG2 cells.[182] The naturally occurring 9-anthrone **143**, obtained from a soil fungus of the genus *Penicillium*, is one of the most active ACLY inhibitors known. Two possible explanations were proposed to account for this remarkable activity: (1) the methylene group,

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activated by the chlorine atom, serves as a site for irreversible reaction with the enzyme, and (2) hydration of the anthrone carbonyl mimics the citrate substrate.[183]

CONCLUSION

The lipogenic pathway has emerged in recent years as a prospective target for anticancer drug design and development, spurred by findings that the three major enzymes of the pathway, FASN, ACC, and ACLY, are up-regulated in numerous tumor types. Moreover, the observation of a correlation between enhanced lipogenesis and tumor aggressiveness and unfavorable prognosis renders the lipogenic pathway as an especially attractive target for antitumor drug design. These observations have been accompanied by several reports that pharmacological inhibition or knock-down of these enzymes can block or reverse tumor progression. Taken together, these reports, along with the recent availability of high-throughput screening assays for all three enzymes, should provide a powerful stimulus to this line of anticancer drug discovery.

However, in spite of the abundance of evidence pointing to the lipogenic pathway as a likely target for cancer, progress to date in this area has been modest at best. This may be due, at least in part, to the lack of consensus on whether lipid overexpression represents a cellular response to the transformed state or is a major factor contributing to it. Furthermore, the lack until recently of crystallographic data on these enzymes has hampered attempts to use molecular modeling as a vehicle for inhibitor design. Additionally, exploration of the pathway as a target for cancer, particularly with reference to ACC and ACLY, appears to have been eclipsed to a degree by the relevance of these enzymes as exceptionally appealing targets for treatment of diabetes and obesity.

Few inhibitors of the lipogenic pathway with antitumor activity have been discovered, with the majority of the work focused on FASN, a most remarkable enzyme capable of catalyzing the synthesis of long chain fatty acids, such as palmitate, from malonyl CoA and acetyl CoA in a series of four repetitive steps. Although FASN inhibitors represent the bulk of known blockers of lipid synthesis, even here, there has been a lack of substantial progress on establishing structure−antitumor activity relationships. The recent publication of mammalian FASN crystal structures complexed with known inhibitors, e.g., **7** and **19**, offers the prospect for increased interest and activity in the design of more specific inhibitors of FASN. The interesting antitumor FASN inhibitor C93, whose structure has yet to be disclosed, represents another promising addition to the list of compounds with clinical potential.

The ACC1 isozyme may be an especially attractive target for antitumor drug development with reduced potential for causing weight loss; however, only a very limited number of compounds with ACC1 selectivity have been identified, none of which have been studied as antitumor compounds. Likewise, although some good inhibitors of ACLY, the third enzyme in this sequence, are known, their antitumor potential remains virtually untapped. Recent publication of the crystal structure of citrate bound to ACLY now provides an opportunity to explore this enzyme as an anticancer target.

In summary, although several decades have passed since the linkage between lipogenesis and tumor development and progression was uncovered, there remains much room for improvement in our understanding of the mechanism(s) coupling these processes. Thus, new anticancer drugs developed and designed to inhibit lipid production, either as primary antitumor agents or as supplements to enhance the antitumor properties of established agents, are approaches to cancer chemotherapy whose time may be at hand.

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ABBREVIATIONS USED

AACT, acetoacetyl-CoA thiolase; ACC, acetyl-CoA carboxylase; ACLY, ATP-citrate lyase; ACS, acyl-CoA synthase; AKR1B10, aldo−keto reductase family 1 member B10; AMP, adenosine monophosphate; AMPK, AMP-dependent protein kinase; ATP, adenosine triphosphate; BRCA1, breast cancer 1; CoA, coenzyme A; CPT, carnitine palmitoyltransferase; ChERBP, carbohydrate response element binding protein; CS, citrate synthase; FASN, fatty acid synthase; HER2, human epidermal growth factor receptor 2; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MAP, mitogen-activated protein; MCD, malonyl-CoA decarboxylase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; OAA, oxaloacetate; PDH, pyruvate dehydrogenase; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PP2A, protein phosphatase 2A; PTEN, phosphatase and tensin homologue; SCD1, stearoyl CoA desaturase 1; SREBP-1c, sterol response element binding protein 1c; TG, triglyceride; VLDL, very low density lipoproteins

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