Drugs of the Future 2003, 28(4): 371-381

CCC: 0377-8282/2003

Review Article

Carnitine palmitoyltransferase inhibitors in the management of type 2 diabetes: an old promise to be maintained

Fabio Giannessi

Department of Endocrinology and Metabolism, Sigma Tau Pharmaceutical Industries S.p.A., Via Pontina Km 30.400, 00040 Pomezia (Rome), Italy

CONTENTS

Abstract

Type 2 diabetes is a complex metabolic disease of epidemic proportions, and is more prevalent in people with a Western life-style. Present treatments consist mainly of insulin secretagogues, metformin and insulin sensitizers. This review will focus on the therapeutic potential of carnitine palmitoyltransferase (CPT) inhibitors, which act on long-chain fatty acids transport into the mitochondria. Particular attention will be devoted to the group of structurally related carnitine inhibitors, where a new selective molecule has now entered clinical development. In the past, a number of compounds have shown interesting characteristics without producing, for several reasons, a clinically available drug.

Introduction

Type 2 diabetes, previously designated non-insulin-dependent diabetes mellitus (NIDDM), is the only noninfectious disease recognized to be of widespread epidemic proportions (1). It presently affects approximately 135 million people, and it is predicted that by the end of this decade there will be 200-300 million cases. The incidence is particularly high in countries with a Western lifestyle (2, 3). Obesity, lack of exercise and excess eating, especially high-fat diets, are the predisposing factors for the development of insulin resistance, the underlying metabolic condition leading finally to high blood glucose levels (4).

Pharmacological interventions aimed at controlling hyperglycemia, and thus preventing serious and life-threatening diabetic complications (retinopathies, cardiovascular accidents, peripheral neuropathies) comprise the use of the insulin secretagogues sulphonylureas (5) and metformin, whose mechanism of action is still under investigation (6, 7), together with the new and controversial class of the insulin sensitizers thiazolidinediones (8, 9).

Sulphonylureas are associated with a risk of severe hypoglycemia (5), partially overcome by new-generation congeners (10, 11), while biguanides like metformin present gastrointestinal side effects (12, 13) and are associated with a risk of lactic acidosis (14). Neither class of compounds are able to stop the progression of the disease, and consequently insulin therapy is normally required. The thiazolidinediones, whose marketed representatives are troglitazone, rosiglitazone and pioglitazone, are a new class of antidiabetic agents with insulin sensitizing activity that have been linked to PPARγ activation. However, there are concerns about hepatotoxicity, edema and cardiac hypertrophy with these agents. Troglitazone, the first to be marketed, was withdrawn following fatal cases of liver toxicity, and the real improvement in the management of the disease attributable to these new pharmacological tools has been recently reconsidered (15).

In addition to the above classes of compounds, alternative or complementary approaches for the treatment of diabetes are presently under extensive investigation, for example, protein tyrosine phosphatase 1B inhibitors (16), β_3-adrenergic agonists (17) and α-glucosidase inhibitors (18). This review will focus on the potential of fatty acid oxidation (FAO) inhibition (19), in particular, inhibition of carnitine palmitoyltransferase (CPT), an old but not completely explored metabolic approach that for several reasons has not yet produced a clinically available compound.

Rationale for CPT I inhibition in type 2 diabetes

Hepatic gluconeogenesis contributes to the fasting and postabsorptive hyperglycemia in type 2 diabetes (20), and this appears to be related to the lack of suppressive effects of both hyperglycemia and hyperinsulinemia on hepatic glucose production. A high level of plasma free fatty acids (FFA) leads to an increase in liver mitochondrial β-oxidation, which drives gluconeogenesis at higher rates (21). As the inner mitochondrial membrane is impermeable to long-chain FFA and their CoA esters, the mitochondrial oxidation of long-chain FFA is made possible by two membrane-bound carnitine-dependent long-chain acyltransferases, also known as carnitine palmitoyltransferases (CPT) (22). CPT I enzyme (located on the outer mitochondrial membrane) catalyzes the formation of long-chain acylcarnitines from their CoA esters, then a translocase transports the FFA carnitine esters across the inner mitochondrial membrane, and finally the CPT II enzyme (located on the inner surface of the inner mitochondrial membrane) reconverts long-chain acylcarnitines into long-chain acyl-CoA esters. These are then β-oxidized to acetyl-CoA, which activates a key gluconeogenetic enzyme, pyruvate carboxylase (23). CPT inhibitors, by lowering the level of acetyl-CoA, indirectly reduce liver gluconeogenesis and could hence be useful in the treatment of type 2 diabetes as antihyperglycemic agents (24). Confirming this hypothesis, in recent years some studies have reported hypoglycemic episodes for patients with genetic CPT deficiencies (25, 26).

Two genes encode for what are known as the liver (L-CPT I) and muscle (M-CPT I) isoforms of CPT I, while CPT II is the product of a single gene. In heart muscle, CPT I isoform is predominantly the same as that in the skeletal muscle, while only a minor component of the liver isoform is present. Consequently, compounds able to inhibit L-CPT I would preferentially affect the liver and only to a small extent the heart, compounds able to inhibit M-CPT I would affect skeletal muscle, heart and adipose tissue, while CPT II inhibitors would affect all tissues.

A focal characteristic for CPT inhibitors as potentially clinically useful antidiabetics is, therefore, the preferential activity on liver with respect to muscle CPT I isoform, and important side effects such as cardiac hypertrophy have actually been observed using nonselective compounds (27). In addition, the selectivity between L-CPT I and CPT II must also be taken into consideration.

Inhibitors at the malonyl-CoA site

Malonyl-CoA is the physiological inhibitor of CPT I (28), and a certain activity has been reported for analogues such as succinyl-CoA and methylmalonyl-CoA (29). Even in the absence of the CoA moiety, some activity is preserved, while the presence of the dicarbonyl functionality is a strict requirement (30). Thus, malonic acid (**1**) and oxalic acid (**2**) (Fig. 1) were shown to inhibit

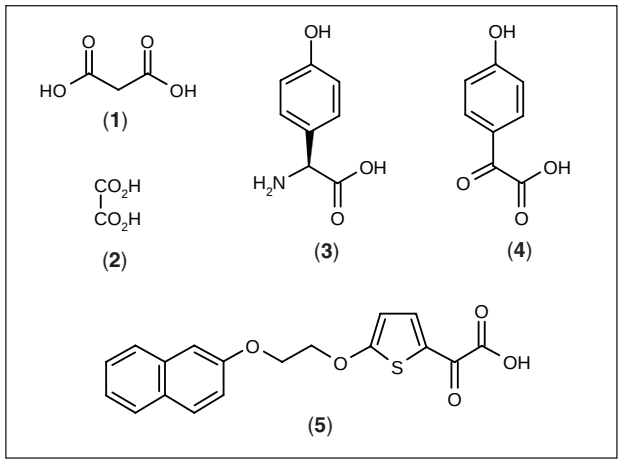

Fig. 1.

CPT I by 15% and 10%, respectively, and inhibition was found with other molecules possessing, or able to generate, such a functional group. Oxfenicine (**3**), which is transformed by transamination to 4-hydroxyphenylglyoxylic acid (HPG) (**4**), has been shown to be able to reduce fatty acid oxidation in heart muscle, and consequently to improve carbohydrate oxidation (31), a condition considered favorable in patients with coronary heart disease (32). In this case, the preferential activity in heart with respect to liver may derive also from the higher levels of transaminase in the cardiac muscle with respect to liver (33). Ro-25-0187 (**5**) and HPG both have been shown to inhibit CPT I at the malonyl-CoA site, and Ro-25-0187 is even more potent than the physiological inhibitor (30). However, two problems arise when considering this site as the target for inhibition. The first is that in conditions of increased FAO, as encountered in diabetes, CPT I activity is increased by 50%, and sensitivity to malonyl-Coa is decreased to 1/10 of the normal values (34). Also of concern is that such inhibitors could affect other metabolic processes involving malonyl-CoA.

Glycidic acids

Glycidic acids such as TDGA (**6**) (tetradecylglycidic acid), clomoxir (**7**) and etomoxir (**8**) (Fig. 2), all of which are structurally characterized by an epoxide moiety, are the more studied and better known CPT inhibitors (35, 36). Their mechanism of action involves the preliminary conversion to the corresponding CoA esters (35), probably followed by reaction of the epoxide functionality to give a covalent bond with the catalytic site, and consequently leading to irreversible inhibition. The stereochemistry of the epoxide, together with its steric environment, is an important feature. The (*R*)-isomer is the active one (37, 38), and substitutions with methyl groups in the 3-position (compounds **9-10**), as well as the transposition of the epoxide group (compound **11**), generated inactive

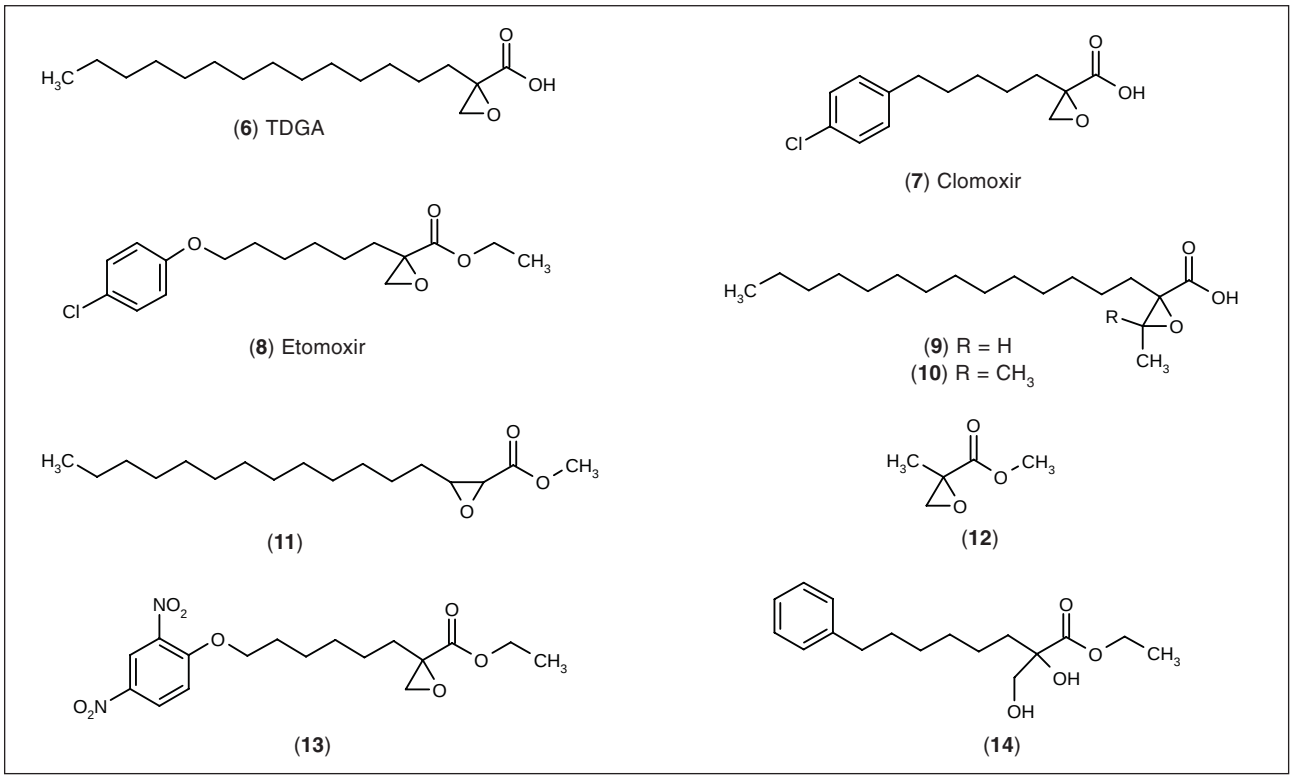

Fig. 2.

molecules (39, 40). Modifications of the carboxylic functionality that allow the formation of the CoA esters, as is the case with methyl and ethyl ester or sodium salts, are tolerated. A scalar range of IC_{50} values has been reported for a series of compounds with increasing chain length, demonstrating that longer alkyl chains give better inhibitory activity, as expected on the basis of CPT I substrate selectivity (39), and methyl 2-methylglycidate (**12**) is inactive.

Glycidic acids confirmed their potential as antiketotic and hypoglycemic agents in animal models of type 2 diabetes, where keton bodies were lowered and hypoglycemic activity was observed, and also in humans (37, 41, 42). In a study in 48 type 2 diabetic patients, etomoxir (12.5, 25 and 50 mg b.i.d. for 14 days) caused a decrease in fasting β-hydroxybutyrate (–50%), glucose (–20%) and triglycerides (–30%). In another clinical study, etomoxir was reported to improve insulin sensitivity (43). This last result is in contrast with that of a recent study in normal rats fed either a low-fat or a high-fat diet, where insulin resistance was found after a 4-week treatment with (*R*)-etomoxir (44). Together with fatty liver and hepatic peroxisomal proliferation, the major concern in preclinical studies with glycidic acids (50 and 200 mg/kg/day of clomoxir for 12 weeks in rats) is myocardial hypertrophy (45). Pathologic cardiac hypertrophy is associated with decreased cardiac function and increased risk of congestive heart failure, sudden death and myocardial infarction (46). The fact that the risk of heart failure is 4-5 times higher in diabetics than in normal subjects (47) may certainly have discouraged continuing the development of drugs with the potential of inducing myocardial hypertrophy as antidiabetics. These effects can be attributed to the inhibition of CPT I, and not to a different mechanism, since bypassing the CPT I system by administration of octanoic acid prevented the development of myocardial hypertrophy (48). These results clearly support the interest in compounds able to selectively inhibit the liver CPT I with respect to the cardiac isoform. Among the glycidic acids, the dinitrophenyl derivative (**13**) of etomoxir was reported to be a selective inhibitor of liver over muscle CPT I (49, 50). Compound **14**, where the oxiranecarboxylic moiety was substituted and mimicked by a 2,3-dihydroxypropionate functionality, was associated with fewer side effects and good antidiabetic activity (51) (Fig. 2). The majority of studies on CPT I inhibitor-induced myocardial hypertrophy were performed in normal animals, and no functional changes of the heart associated with hypertrophy were observed. In diabetic animals, where a precondition of cardiac dysfunction is present, CPT I inhibition has resulted in favorable effects on cardiac function (52-56).

Etomoxir, probably discontinued as an antidiabetic due to its effects on cardiac muscle, was also under clinical development in patients with congestive heart failure (NYHA class II or III symptoms), with early reports indicating positive effects on overloaded heart muscle, associated with improved function, attributed to selective

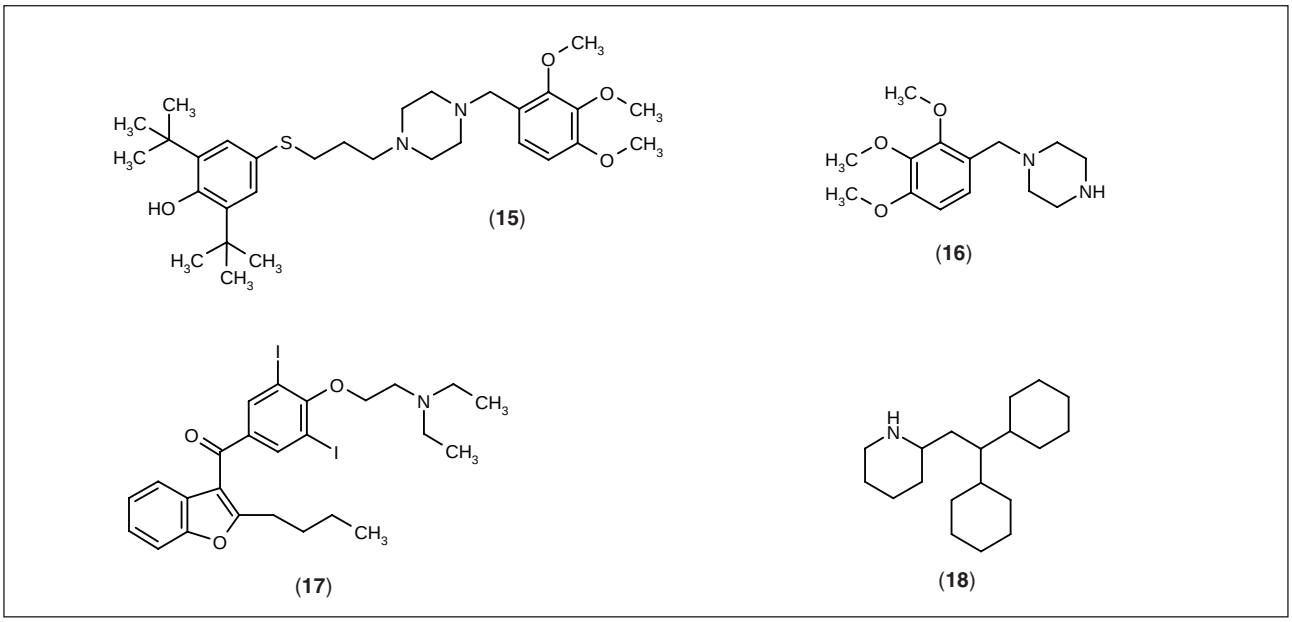

Fig. 3.

changes in the dysregulatory gene expression of hypertrophied cardiomyocytes (57, 58). However, in June 2002, development of the drug for the treatment of heart failure was discontinued due to results from a phase II clinical trial indicating that it did not have the anticipated efficacy in this indication (59). The structurally unrelated compound S-15176 (**15**) (Fig. 3), a derivative of the antianginal drug trimetazidine (**16**), was recently reported to be a more effective CPT I inhibitor *in vitro* in heart (IC_{50} = 16.8 μM) than in liver mitochondria (IC_{50} = 50.8 μM), and was also effective *ex vivo* in heart and liver tissues after a 2-week treatment. This inhibitory effect may shift heart metabolism from fatty acid to glucose oxidation, contributing to the antiischemic effects of the drug (60). A weaker CPT I inhibiting activity was previously reported for two other agents with proven antianginal effects, amiodarone (**17**), which displayed an IC_{50} value of 228 μM for cardiac mitochondria, and perhexiline (**18**), which displayed IC_{50} values of 77 and 148 μM for cardiac and hepatic mitochondria, respectively (61). Again, this action was considered likely to contribute to the antiischemic effects of the drugs.

Carnitine related substrate inhibitors

Several molecules structurally related to carnitine were identified early as inhibitors of the CPT system. Among the first, palmitoyl and decanoyl (*S*)-(+)-carnitine (compounds **19** and **20**, respectively, Fig. 4) (62-64), enantiomers of the physiological acyl (*R*)-(–)-carnitine, were found to behave as competitive reversible inhibitors, reversing diabetic ketoacidosis when infused in alloxan diabetic rats (65). Since they can undergo degradation to give (+)-carnitine, and therefore inhibit other carnitine-dependent enzymes such as carnitine acetyltransferase (CAT) (66), they were soon abandoned. Several years later, McGarry *et al*. elucidated the true mechanism by which medium- and long-chain (+)-acylcarnitines inhibit mitochondrial fatty acid transport. Contrary to long-standing belief, they established that they inhibit carnitine acylcarnitine translocase (CACT, inner membrane) rather than CPT I or CPT II (67).

Aminocarnitine and a series of acyl derivatives were discovered, synthesized and investigated as more stable analogues. Aminocarnitine, believed at first to be a CPT I inhibitor (68), was shown to be more selective for CPT II (69-72), with IC_{50} values of 0.8 and 19 μM against CPT II and CPT I, respectively (71). (*R*)-Aminocarnitine **21** (Fig. 4) is a more potent inhibitor than the (*S*)-enantiomer (68). The length of the acyl moiety in its derivatives influences the activity, with long-chain acylaminocarnitines inhibiting CPT I while short-chain acylaminocarnitines inhibit CAT (73). Since (*R*)-aminocarnitine may replace (*R*)-carnitine as a substrate, forming acetylaminocarnitine and therefore inhibiting CAT and possibly other carnitine-dependent enzymes, this would obviously create a problem. It is less clear why palmitoylaminocarnitine (**22**) has not been successfully developed (74), although a lack of enzyme specificity has been hypothesized. Furthermore, the potential to give aminocarnitine after chemical and/or enzymatic degradation, with the consequent indirect non-specificity, is also present. This point is discussed below.

A somewhat different series of carnitine analogues is represented by molecules designed as transition state analogue inhibitors (75, 76), but evidence that they do not behave merely as substrate analogues is not available. Hemipalmitoylcarnitinium (HPC) (**23**) (Fig. 4), reported by

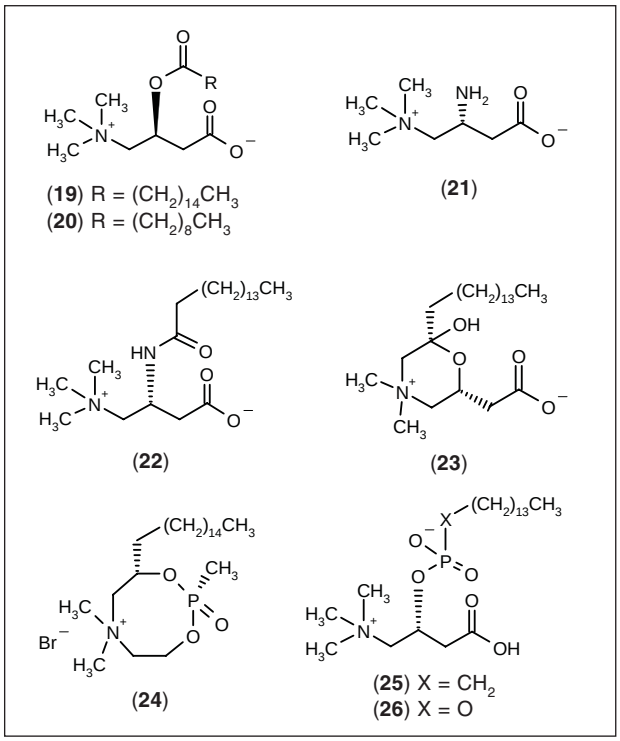

Fig. 4.

Gandour *et al.*, showed a K_i of 2.8 μM for rat heart but only 4.2 μM for liver CPT I (77), while racemic HPC showed a K_i of 5.1 μM for CPT II (78). Gandour also reported the cyclic phosphate **24**, with an IC_{50} of 128 μM for CPT II (79). Based again on transition state analogue design theory, scientists from Sandoz described a series of phosphonate and phosphate inhibitors, with compound **25** and SDZ-CPI-975 (**26**) showing IC_{50} values of 3.0 and 3.4 μM, respectively, in the production of keton bodies from oleate in hepatocytes from rats fasted for 18 h (80). Palmitoylaminocarnitine (**22**) had an IC_{50} of 1.3 μM in the same test, but quite surprisingly the phosphonamidate **27** was inactive, and the phosphoramidate **28** was less active (IC_{50} = 24 μM). (*R*)-Enantiomers were confirmed to be the most active ones, and the influence of substituents in the acyl chain was investigated, leading to compounds such as SDZ-CPI-267-597 (**29**) (Fig. 5) with higher potency (IC_{50} = 93 nM) (81, 82). Greater potency was also observed with the thiophosphate derivative **30** (83), demonstrating again the importance of the functional group linking the carnitine skeleton and the acyl chain.

SDZ-CPI-975 was developed preclinically and proven to be an orally active, liver selective CPT I inhibitor *in vivo* (84, 85). However, it was discontinued under clinical phase I because hepatic mitochondrial abnormalities were observed in normal treated rats. These abnormalities were also observed with etomoxir, but not with the inactive enantiomer of SDZ CPI 975. Although the reported abnormalities might have been due to CPT I inhibition, Anderson comments that "the clinical consequences of these changes are not clear, as there was no evidence of hepatocellular necrosis", and also that "hepatotoxicity is also seen in normal animals, but question is open as to the outcome that one could find in the disease state" (74).

In the case of SDZ-269-456 (**31**) (Fig. 5), an acyl derivative of a cyclic structure mimicking aminocarnitine, an IC_{50} of 19 μM for inhibition of FAO in hepatocytes from 18 h fasted rats was found (81, 82). Between cyclic structures, a series of stereoisomers of carnitine derivatives were reported, with compound **32** shown to be an inhibitor of CPT I able to reduce enzymatic activity by 94% at 500 μM (86). Racemic **33** is another cyclic structure reported to be a competitive inhibitor of neonatal rat cardiac myocyte CPT I (K_i = 0.5 mM, K_m = 0.2 mM for (*R*)-carnitine) and a noncompetitive inhibitor of neonatal

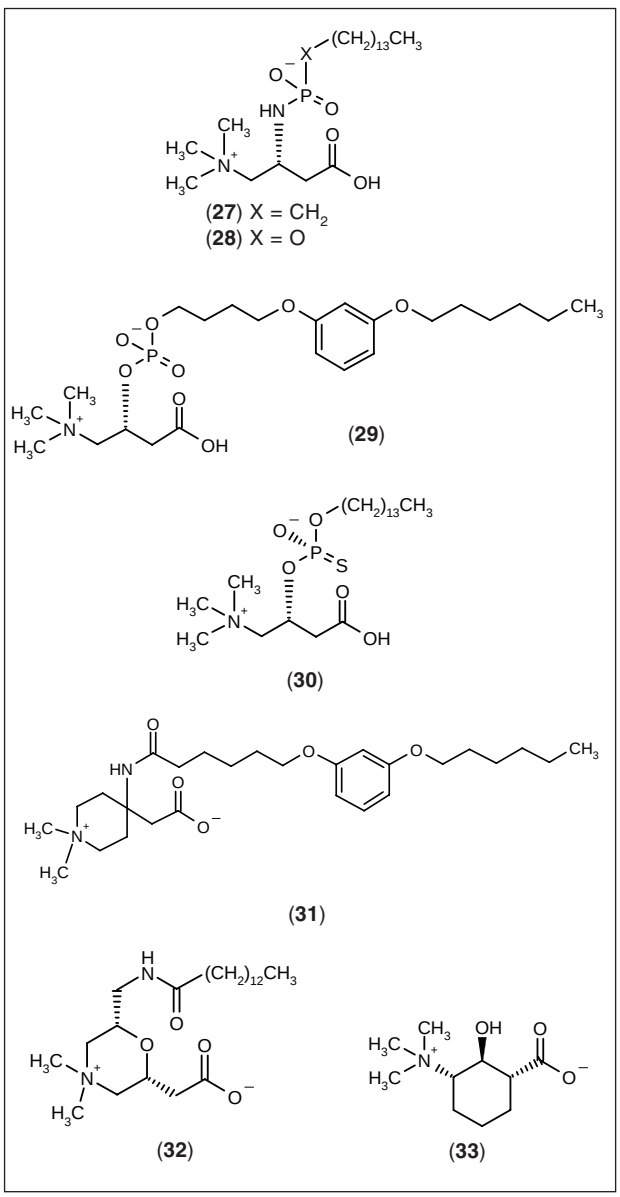

Fig. 5.

rat cardiac myocyte CPT II (K_i = 0.67 mM) (87), suggesting that the compound represents the bound conformation of (*R*)-carnitine for CPT I. The substitution of the skeleton of the physiological molecule carnitine with that of cyclically constrained analogues, even if it has not led to more active compounds, has given insights into the conformational characteristics of the bound ligand.

The investigation of the effects of substitutions of the functional groups of carnitine with analogues (for example quinuclidinium, pyridinium or trimethylphosphonium in the place of trimethylammonium, and phosphonate or tetrazole in the place of the carboxylic function) was carried out in a multivariate approach study including several functional groups as linkers between the "carnitine" skeleton and long or medium alkyl chains (88). It was found that substitutions in the carnitine moiety led to loss or substantial lowering of activity, and that derivatives of (*R*)-aminocarnitine were more active than those of carnitine. Compounds **34** and **35** (Fig. 6) showed IC_{50} values of 0.8 μM and 0.7 μM, respectively, with a 7- and 5-fold selectivity for the liver isoform. Compound **35** showed a 28% reduction of blood glucose levels in *db/db* diabetic mice when administered orally (50 mg/kg twice daily) for 30 days.

An ureido derivative, ST-1326 (**36**), showed an even better profile, with an IC_{50} of 1 μM, and a comparable blood glucose lowering effect in the same *in vivo* model, but with an extraordinarily high selectivity (40/1) for the liver with respect to the muscle isoform (89). ST-1326 was also reported to be able to potently reduce β-hydroxybutyrate levels when orally administered to 24-h fasted rats (ED_{50} = 14.5 mg/kg). In a small ureidic series, the scalar activity found *in vivo* in *db/db* mice reflected the *in vitro* results, with the longest alkyl chain giving the highest inhibition. Furthermore, ST-1326 showed selectivity for CPT I over CPT II, much higher than the structurally related (*R*)-palmitoylaminocarnitine, as evidenced in a test with oleate in hepatocytes. In this test, the levels of oleylcarnitine were about 10-fold higher than control values after treatment with (*R*)-palmitoylcarnitine, demonstrating a strong inhibitory effect for this molecule on the inner membrane isoform (CPT II), while no elevation was observed after treatment with ST-1326. It was also hypothesized that, given the chemical stability of the ureidic function confirmed for ST-1326 in aqueous basic or acidic conditions mimicking stomach and gut conditions (pH = 2 and pH = 8.5, respectively), and the low predictable risk of enzymatic hydrolysis, the possibility for aminocarnitine to be formed is very low. On the contrary, palmitoylaminocarnitine was more prone to undergo hydrolysis, with its concentration beginning to decrease in both conditions just after 2 h. ST-1326 was selected for development as potential antiketotic and antidiabetic drug.

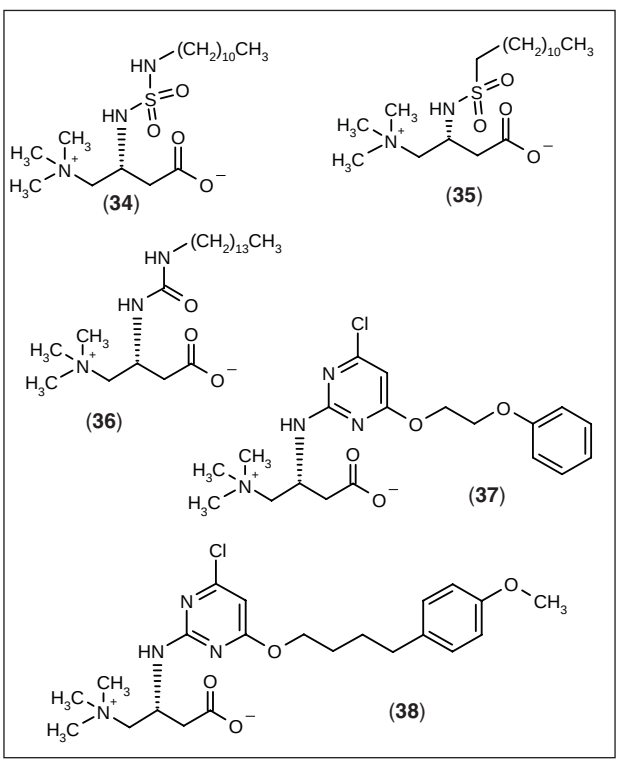

Fig. 6.

Related structures were reported in a patent where the CPT inhibitory activity of molecules such as **37** and **38** (Fig. 6) was claimed (IC_{50} = 30 μM) (90). The activity was lower than that of the previously reported compounds **26**, **29**, **30** and **34-36**; nevertheless, the presence of an heteroaromatic ring directly linked to the amino group of (*R*)-aminocarnitine introduces an interesting element of novelty, while the presence of the alkoxyphenyl group confirms to a certain extent what was already observed in the case of compound **29**.

Miscellaneous CPT inhibitors

Some molecules belonging to different therapeutic classes have been reported to behave as CPT inhibitors. The sulfonylurea insulin secretagogue glyburide (**39**) (Fig. 7) is an aspecific liver and muscle inhibitor, with an IC_{50} of 40 μM for total liver CPT activity, while another sulfonylurea, tolbutamide (**40**), has an IC_{50} of only 1400 μM (91). Bezafibrate (**41**), a representative of the class of hypolipidemic fibrates in part structurally related to glycidic acids, has been proven to inhibit CPT I in rat isolated hepatocytes up to 44% at 100 μM (92). The tetrazoles 4-THA (**42**) and LY-171883 (**43**) (93, 94), fibrate analogues, showed approximately 70% inhibition of CPT I activity at 100 μM, and 4-THA has been proven unable to inhibit CPT II. A slight inhibition for both CPT I and CPT II was also reported in the case of adriamycin (**44**), an antineoplastic agent and glycoside antibiotic with cardiotoxic side effects (95, 96), and for the antileukemic agent mitoguazone (**45**) (97), structurally reminiscent of the antidiabetic metformin. The variety of structures able to interact with CPT, even if at quite high concentrations, opens a range of possibilities to the design and investigation of new-generation inhibitors.

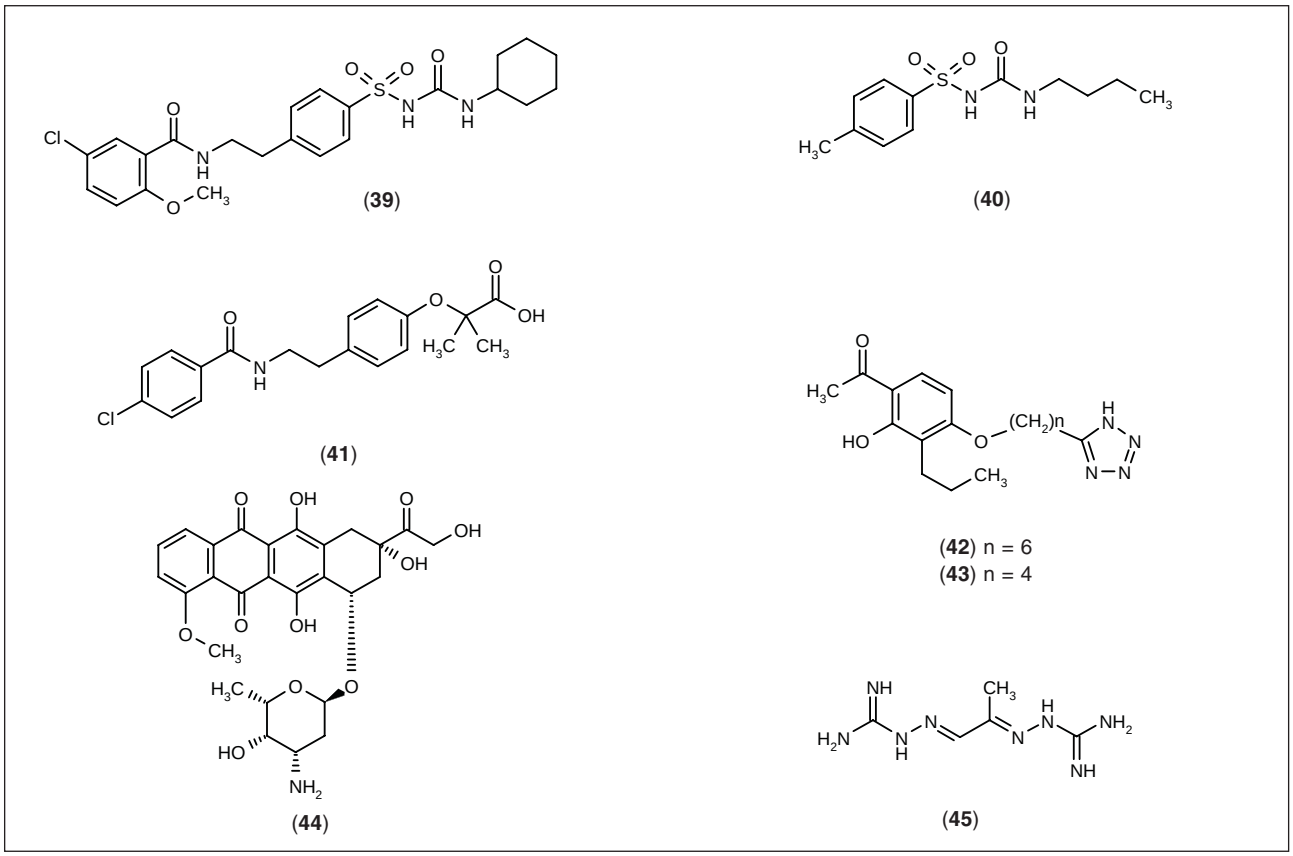

Fig. 7

CPT II inhibitors

We have stated before that one of the conditions for CPT inhibitors to be potentially useful in the treatment of metabolic diseases, such as diabetes, is that they behave as selective inhibitors of the liver isoform of CPT I, with respect to the ubiquitous CPT II. Nevertheless, it has been reported that CPT II inhibitors that act by sequestering intramitocondrial CoA, for example compound **46** (Fig. 8), may become selective for the liver by means of prodrug systems as reported for compounds **47** and **48** (98, 99). In the case of **48**, for example, the ester with glycerol will be concentrated in the liver and transformed into the active compound (**46**). It is too early to determine

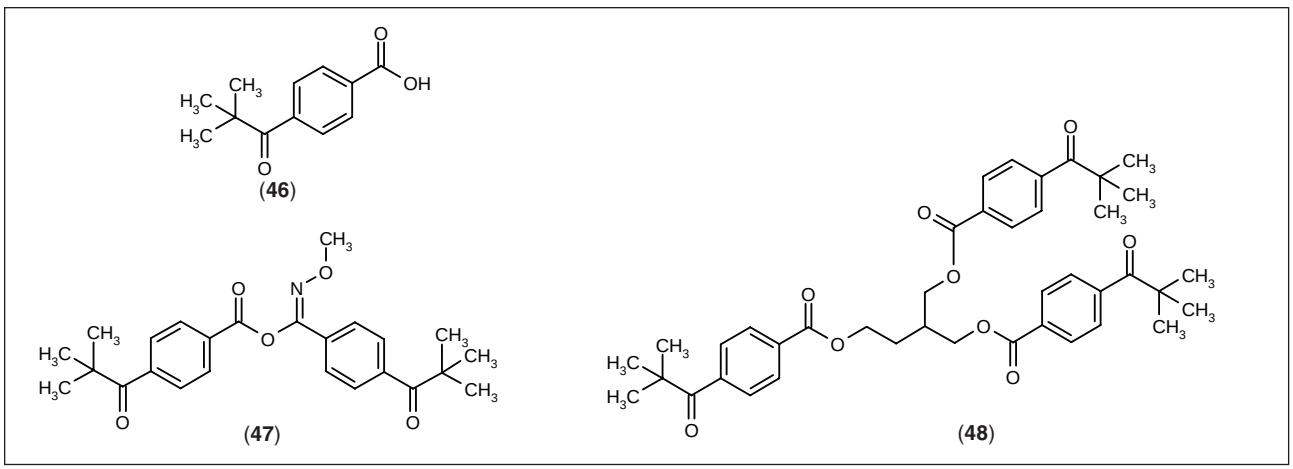

Fig. 8.

if this renewed interest in inhibition of CPT II will be followed by future research and/or clinical development, but concern arises from the possible concomitant inhibition of CAT and other carnitine-dependent enzymes.

Conclusions

Inhibition of fatty acid oxidation with inhibitors of the carnitine-acyl transferases system, particularly CPT I, has been widely studied as a strategy for lowering blood glucose levels in type 2 diabetes. After the clinical discontinuation of glycidic acids because of their mechanism-based myocardial hypertrophy, and of SDZ-CPI-975, reported to induce hepatic mitochondrial abnormalities, ST-1326, a new selective and reversible CPT I inhibitor, is now undergoing clinical trials. It certainly represents a good opportunity for finally understanding the real potential and the toxicological concerns of this class of compounds, renewing the hope that the old promise of managing diabetes with CPT inhibitors, alone or in combination with other antidiabetic agents, may finally be fulfilled.

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