Expert Opinion

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Novel microsomal triglyceride transfer protein inhibitors

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Microsomal triglyceride transfer protein (MTP) mediates triglyceride absorption and chylomicron secretion from the intestine and very-low-density lipoprotein (VLDL) secretion from the liver, by linking lipid molecules with apolipoprotein B (ApoB). Inhibition of MTP reduces the level of all ApoB-containing lipoproteins, including low-density lipoprotein (LDL). High-throughput screening has produced several families of compounds that are effective *in vitro* and *in vivo* as MTP inhibitors and some drugs are currently at clinical trial stage. Drugs that inhibit MTP can potentially be very effective in reducing atherosclerotic vascular disease by lowering levels of all the atherogenic lipoproteins. Partial inhibition of MTP by an inhibitor could be particularly useful when combined with other drugs that alter lipid metabolism but marked inhibition of MTP could cause significant adverse effects.

Keywords: apolipoprotein B (ApoB), chylomicron, microsomal triglyceride transfer protein (MTP), triglyceride, very-low-density lipoprotein (VLDL)

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1. Introduction

Atherosclerosis is a major cause of mortality and morbidity in countries with a high consumption of cholesterol and saturated fat [1]. Therapeutic intervention to reduce atherosclerosis has focused on drugs that lower cholesterol by reducing intestinal absorption of cholesterol or inhibiting hepatic synthesis of cholesterol. However, reduction of intestinal absorption and hepatic production of triglyceride could also play a major role in the reduction of atherosclerosis and cardiovascular disease. There is compelling evidence that hypertriglyceridaemia is a risk factor for cardiovascular events [2] and the combination of elevated triglyceride and elevated cholesterol to high-density lipoprotein (HDL) cholesterol ratio forms a particularly adverse lipid profile [3].

Microsomal triglyceride transfer protein (MTP) mediates triglyceride absorption from the intestine and secretion from the liver. This review focuses on recently patented compounds that inhibit MTP and therefore have the therapeutic potential to reduce triglyceride levels more significantly than currently available therapy. These compounds also have the potential to promote weight loss by reduction of fat absorption and to reduce cholesterol levels. MTP inhibitors could be used advantageously in combination with other pharmaceutical compounds that reduce lipid levels, enhance insulin sensitivity or promote weight loss. There are significant potential adverse effects from excessive inhibition of MTP, including fat malabsorption with vitamin deficiency and fatty infiltration of the liver.

2. Lipid metabolism

The lipid transport pathway starts with the formation of triglyceride-rich particles, either from intestinal absorption of dietary and biliary fat or from secretion by the liver [4,5]. Each of these particles contains one copy of the protein ApoB, which has the capacity to bind triglyceride and dock with hepatic receptor

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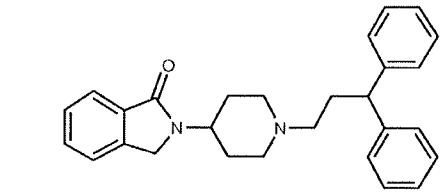

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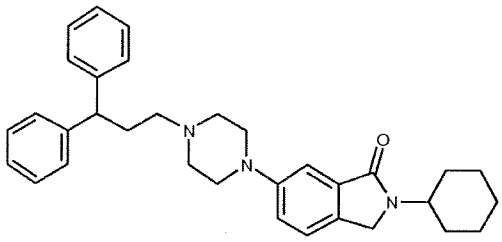

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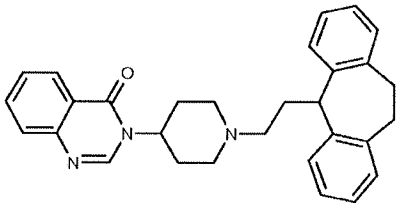

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proteins after delipidation. Very-low-density lipoprotein (VLDL) particles of hepatic origin contain the 4,536 amino acid ApoB-100, whereas chylomicrons and VLDL of intestinal origin have the truncated 2,152 amino-terminal section of ApoB-100, known as ApoB-48 [6]. After a lipid-rich meal there is a postprandial rise in triglycerides, due to an elevation of chylomicrons. Low-density lipoprotein (LDL) is the major cholesterol transport lipoprotein particle and arises from the delipidation of VLDL by the action of peripheral lipases. It is cleared from the circulation through binding to LDL-receptor proteins, predominantly in the liver. Lipoprotein (a) is similar to LDL but also contains Apo(a), which has close homology to plasminogen [7]. Secreted by the liver, lipoprotein (a) is cleared by a mechanism considered to be mainly independent of binding to the LDL-receptor [8].

3. Adverse effects of dyslipidaemia

Increased LDL cholesterol is a major positive predictor of atherosclerotic vascular disease [9] and mutations of the LDL-receptor typically cause marked elevation of LDL-cholesterol and increased atherosclerosis in heterozygotes [10]. Lipoprotein (a) levels are largely genetically determined and high levels have been associated with accelerated atherosclerosis [11]. Triglyceride-rich lipoprotein particles are also potentially atherogenic, particularly chylomicron remnant and VLDL remnant particles and intermediate density lipoprotein (IDL) [12].

The most common genetically determined lipid disorder that results in premature coronary heart disease is familial combined hyperlipidaemia [13]. Manifested phenotypically as variable elevation of cholesterol and triglyceride levels, the main abnormality of lipid metabolism is increased production of ApoB-containing particles (VLDL) from the liver [14].

Elevated fasting triglyceride is commonly associated with insulin resistance and other lipid changes, such as postprandial hyperlipidaemia and small, dense LDL particles that are relatively triglyceride-rich. These abnormalities are particularly prominent in Type 2 diabetes. There is compelling evidence that this pattern of abnormal lipid metabolism carries increased risk of cardiovascular disease [15,16]. Currently, these lipid abnormalities can be treated with a peroxisome proliferator-activated receptor-α (PPAR-α) agonist, such as fenofibrate [17].

4. Microsomal triglyceride transfer protein

The intracellular assembly of chylomicrons in the small intestine or VLDL particles in the liver requires the linkage of triglyceride with ApoB, an interaction mediated by MTP [18]. Situated in the rough endoplasmic reticulum and the Golgi apparatus [19], MTP is a heterodimer, composed of a unique 97 kDa subunit and a smaller disulfide isomerase. The interaction between MTP and ApoB involves independent ApoB binding and lipid transfer functions, both related to the 97 kDa subunit of MTP [20]. MTP catalyses transport between membranes of cholesteryl esters and phospholipids, as well as triglycerides [21]. The disulfide isomerase subunit holds the larger subunit in a stable, active configuration [22].

The first step in triglyceride-rich lipoprotein assembly is the co- and post-translational lipidation of ApoB, which is dependent on MTP. Both MTP and ApoB have homology to the egg yolk storage protein lipovitellin, which forms a homodimer with a lipid-binding cavity. In an analogous way, a lipid transfer cavity can be formed by the association of ApoB with MTP [23]. Subsequent attachment of further lipids and assembly of triglyceride-rich lipoproteins is regulated by phospholipases and acyl cholesterol acyl transferase (ACAT) in a smooth membrane compartment [24].

Expression of the MTP gene is regulated partly through a sterol regulatory binding element (SRBE) in the promoter and is enhanced by cholesterol [25]. Insulin and certain flavonoids have been shown to suppress the MTP gene. In heterozygous MTP knockout mice, ApoB levels were reduced by 28% but the defect was lethal in homozygotes [26]. Mutation of the part of the gene coding for the 97 kDa subunit can result in the autosomal recessive condition of abetalipoproteinaemia, which is characterised by marked reduction of plasma levels of ApoB-containing lipoproteins [27]. Clinical manifestations of this condition (and potential effects of excessive therapeutic inhibition of MTP) are fat malabsorption with severe deficiency of

fat-soluble vitamins, hepatic and intestinal steatosis, acanthocytosis, retinitis pigmentosa and spinocerebellar degeneration [28]. At least one polymorphism in the promoter region of the MTP gene has been associated with lower plasma LDL cholesterol levels [29].

5. Techniques for experimental assessment of microsomal triglyceride transfer protein inhibitory activity

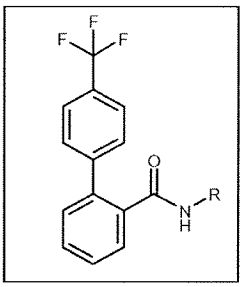
Techniques used for assessment of the therapeutic potential of patented compounds as inhibitors of MTP range from *in vitro* cell-free studies of enzyme activity to clinical trials. MTP can be isolated from hepatic microsomes and its activity determined in cell-free preparations as the ability to transfer radiolabelled triglyceride or phospholipids from donor to acceptor small unilamellar vesicles [30]. Another frequently used *in vitro* assay relies on the ability of MTP inhibitors to reduce secretion of ApoB (but not ApoA1 or albumin) from cultured HepG2 liver cancer cells [30]. This assay can also be performed after transfection of liver cells with ApoB-100 minigenes [20]. The triglyceride content and therefore density of secreted lipoprotein particles depends on the fatty acid content of the medium and the endogenous lipase activity [31]. *In vivo* studies in animals and humans frequently use reduction of plasma cholesterol, triglyceride and ApoB concentrations as the end point.

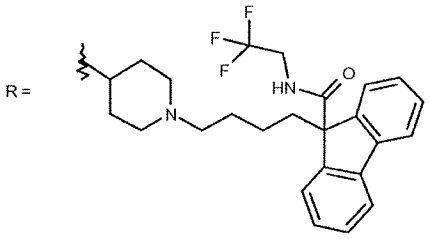
6. Inhibitors of microsomal triglyceride transfer protein

Compounds that inhibit MTP activity could act to inhibit the binding of ApoB or to inhibit the lipid transfer activity of MTP. There is evidence that different compounds may act at the different sites involved in these MTP actions [32]. When MTP activity is inhibited, there is increased degradation of ApoB, partly by the chymotrypsin-like catalytic activity of proteasomes [33]. Because of incomplete knowledge about the structure of MTP, efforts aimed at the discovery of inhibitors have relied heavily upon the use of high-throughput screening [34]. Independent efforts by many groups have given rise to several families of MTP inhibitors that show good promise *in vitro* and *in vivo*. Initially, many of these compounds were developed as inhibitors of ApoB secretion and were later found to reduce secretion of ApoB through inhibition of MTP.

6.1 Isoindolones and quinazolones

Bristol-Myers Squibb identified the isoindolone (compound 1), with an IC_{50} for inhibition of bovine MTP-mediated transport of triglyceride between liposomes of 0.6 µM [30]. Compound 1 also inhibits human MTP-mediated triglyceride transfer (IC_{50} = 2.2 µM) but has little activity in animal models [35]. More recently, Warner-Lambert has described the use of compound 1 to lower plasma





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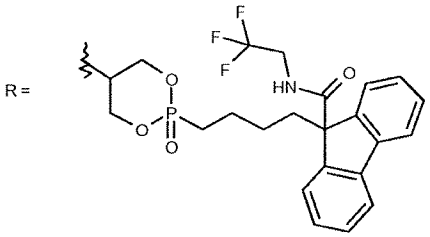

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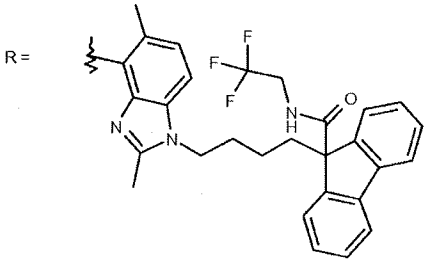

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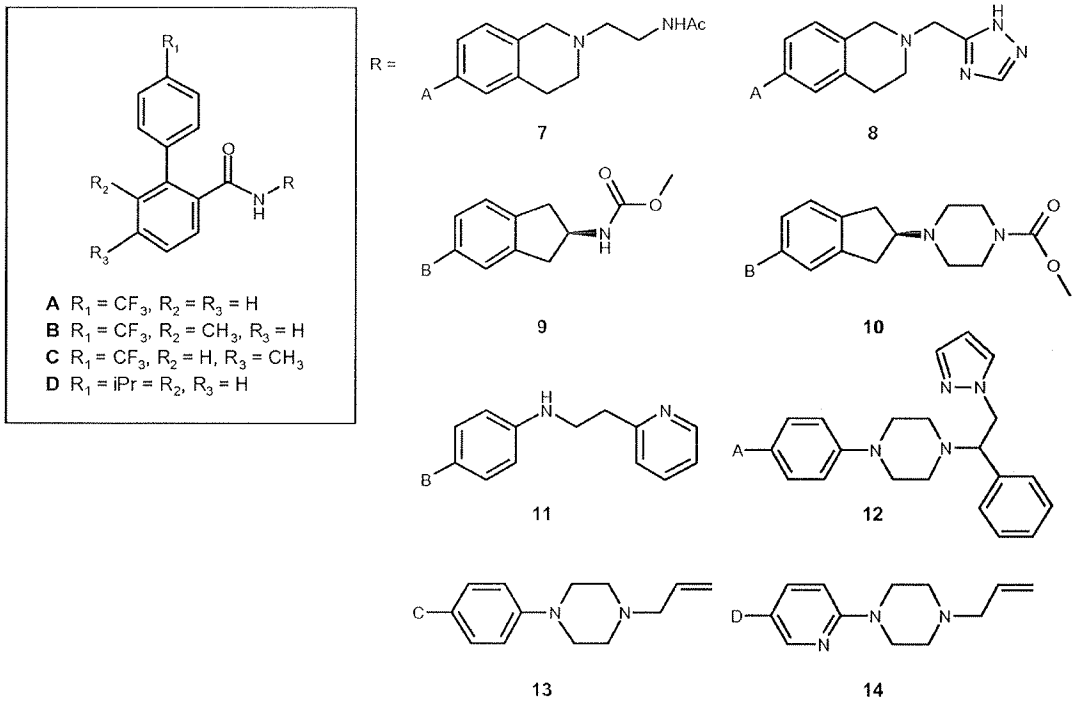
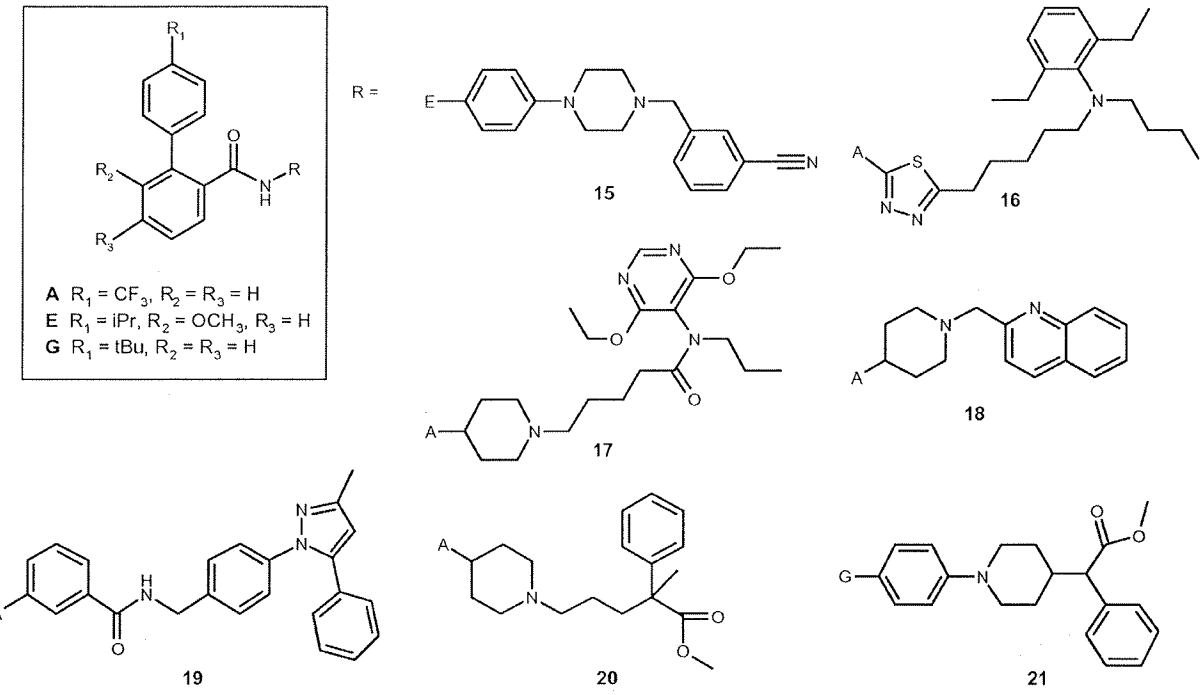
lipoprotein (a) through the inhibition of MTP [101]. Compound 1 lowered the level of lipoprotein (a) secretion from HepG2 cells transfected with human ApoB by 85% at a concentration of 74 µM. Meiji Seika Kaisha has claimed a series of isoindolones including compound 2 [102]. This compound shows 68% inhibition of ApoB secretion from Hep2G cells and inhibits triglyceride production by 89%. Japan Tobacco has disclosed a series of quinazolones, including compound 3 [103]. These compounds were effective inhibitors of MTP-mediated lipid transfer and ApoB secretion from HepG2 cells (IC_{50} = 0.1 and 0.02 µM, respectively).



6.2 2-Phenylbenzamides

Building on leads obtained through high-throughput screening efforts, Bristol-Myers Squibb modified and optimised compound 1 through automated organic synthesis [35,104,105]. In particular, the isoindolone of compound 1 was found to be readily replaced by a benzamide linkage and a fluorenyl group was found to be an excellent replacement for the diphenylmethyl group of compound 1. These studies lead to the development of the 4′-CF_3-phenylbenzamide, BMS-201038 (compound 4). Compound 4 possesses great potency in the MTP-mediated lipid transfer assay (IC_{50} = 0.5 nM) and inhibits ApoB secretion (ED_{50} = 0.8 nM). This compound inhibited acute lipoprotein secretion in both fasted and fed rats, indicating that it inhibits both hepatic and intestinal lipoprotein secretion. In hamsters treated with 6 mg/kg for 7 days fasting, cholesterol level was reduced by 90% and triglyceride by 49%, compared with control animals. The ED_{50} value for cholesterol lowering was 2.4 mg/kg [35]. In Watanabe-heritable hyperlipidaemic (WHHL) rabbits, which are a model of homozygous familial hypercholesterolaemia, treatment with 10 mg/kg of this compound for 14 days normalised plasma lipoprotein levels, reducing cholesterol by 89% and triglyceride by 81% [35], and it has now entered clinical trials. Compound 4 has also been claimed for use in combination with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, such as pravastatin, for lowering serum lipid levels [106], for the treatment of atherosclerosis [107] and for treating lipase deficiencies [108]. Bristol-Myers Squibb has since reported several modifications to this system, including the installation of a phosphonate replacing the piperidine of compound 4 (e.g., compound 5), but no biological data has been disclosed [109]. More recently, Bristol-Myers Squibb has reported optimisation of the linker of compound 4 with the aim of removing the piperidine moiety of concern, owing to the ubiquitous presence of piperidines as ligands for G-protein-coupled receptors. This change resulted in the development of the benzimidazole-linked BMS-212122 (compound 6), a potent inhibitor of triglyceride transport (IC_{50} = 1 nM) and of ApoB secretion from HepG2 cells (IC_{50} = 0.03 nM) [36]. This compound was also tested in a hamster and a monkey model. Treatment of hamsters for 3 days with 1 mg/kg reduced cholesterol levels by 74% and triglyceride by 57% (ED_{50} value for cholesterol 0.28 mg/kg). In cynomolgus monkeys, the same dose reduced cholesterol by 73% and triglyceride by 71% (ED_{50} value for cholesterol = 0.38 mg/kg) [36].

Pfizer has also developed a range of derivatives based on the 2-phenylbenzamide core [110-113]. These inhibitors include the tetrahydroisoquinoline compounds 7 and 8 [114,115]. A series of compounds were tested as antiobesity agents through their ability to reduce the food intake of male beagles when administered at 0.5 mg/kg on days 0, 5 and 12 of a 14-day test period. Compound 7 was reported to reduce food intake by 58% and to reduce intestinal fat absorption by 49% [116,117]. Compound 7 was also claimed to act synergistically with antiobesity agents, including sibutramine in a rat model, but no biological data have been reported [118]. Pfizer has reported that administration of compound 8 to human subjects reduced total serum cholesterol and LDL-cholesterol by 40% and 80%, respectively, over a 17-day treatment period [119]. Novartis has developed



a range of indane-based inhibitors of MTP [37]. These compounds were derived from lead compounds discovered independently through high-throughput screens, first for inhibition of ApoB secretion and then for inhibition of MTP-mediated triglyceride transfer. These compounds are similar to the inhibitors of Bristol-Myers Squibb and Pfizer through the presence of a 2-phenylbenzamide moiety but differ by the presence of an indane ring [120]. In particular, compound **9** was found to inhibit ApoB secretion from HepG2 cells (IC_{50} = 0.7 nM) and inhibit MTP-mediated transfer of triglycerides (IC_{50} = 70 nM) [121]. Compound **10** was also reported as an inhibitor of ApoB secretion and MTP-mediated lipid transfer activity with IC_{50} values of 1.8 and 40 nM, respectively [122]. The indane ring of compounds **9** and **10** can also be replaced by a simpler aryl group. Thus, compound **11** inhibited ApoB secretion from HepG2 cells and MTP-mediated triglyceride transfer, with IC_{50} values of 1 and 90 nM, respectively [123]. Compounds **9** and **10** have been studied in normolipidaemic rats and dogs [37]. At 2 and 6 h after a single dose of 5 mg/kg, compound **9** was reported to effectively lower plasma total cholesterol (30 – 45%) and triglyceride (60 – 80%) in rats. A dose of 50 mg/kg of compound **10** was required to achieve similar reductions. In dogs treated with 5 mg/kg of compound **9** for 4 days, plasma triglycerides fell by 75% and cholesterol by 56%, while compound **10** at 10 mg/kg achieved reductions of 78% and 70%, respectively. Compound **9** has also been studied in LDL-receptor deficient mice. Treatment for 7 days reduced cholesterol levels by 85% and triglyceride by 65%. ApoB-100 levels fell by 70% and ApoB-48 by 80% [38].

Glaxo Group reported the preparation of a series of 2-phenylbenzamide derivatives including compounds **12** – **14** [124-126]. These compounds were tested for inhibition of MTP-mediated transfer of tritiated triolein between liposomes and for inhibition of ApoB secretion, although only limited biological data were reported. The nitrile compound **15** was prepared and showed excellent inhibition of MTP-mediated triolein transfer (IC_{50} = 0.1 nM) [127].

Wakunaga Pharm. Co. has reported a series of aniline derivatives that act as MTP inhibitors. Compound **16** was an excellent inhibitor of MTP (IC_{50} = 0.39 nM) and also inhibited ApoB secretion from HepG2 cells [128]. Modifications to compound **16** afforded compound **17**, which is an extremely potent inhibitor of ApoB lipoprotein secretion (IC_{50} = 0.0029 nM) [129]. Merck has also developed a range of 2-phenylbenzamides, including the piperidinyl quinoline compound **18**. These compounds were tested for their ability to inhibit MTP and ApoB secretion *in vitro* and had IC_{50} values of 26nM and 2 nM, respectively [130]. Boehringer-Ingelheim modified the western end of compound **4** to afford a range of 2-phenylbenzamides, including compounds **19** and **20** [131,132]. No specific biological data were reported for these compounds. In a series of patents, Janssen has disclosed a series of 2-phenylbenzamide derivatives [133,134]. The most potent of these, compound **21**, exhibited MTP inhibitory activity with an IC_{50} of 2 nM [135].

Tanabe Seiyaku has prepared a series of 2-arylbenzamides as inhibitors of ApoB secretion. Thus, a series of compounds including the azaisoindole, compound **22**, and its pyridine congener, compound **23**, were prepared [136,137]. No biological

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