on which they feed. Carboniferous odonatoids were already predatory (*8*), but no contemporary insects would have approached the maneuverability of many extant species. The relatively smaller, less sturdy bodies and consequent lower wing-loadings of Eugeropteridae indicate lower maximum speeds than in Anisoptera, and the absence of a nodus suggests a poorer capability for wing twisting, truncating the lower end of their speed range: It is unlikely that they could hover like modern dragonflies. Nonetheless, the flexible trailing edge and the shortened subcostal vein indicate that some supinatory twisting was possible, and the group appears to be following a trend, parallelled in many other insect groups, toward improving flight versatility by recruiting upstroke forces to supplement those of the far more effective downstroke, and varying their magnitude and direction at need. The "smart" wing-base mechanism is best interpreted as an elegant means of maintaining downstroke efficiency in the presence of these adaptations to improve upstroke usefulness.

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1 June 1998; accepted 22 September 1998

An MTP Inhibitor That Normalizes Atherogenic Lipoprotein Levels in WHHL Rabbits

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Patients with abetalipoproteinemia, a disease caused by defects in the microsomal triglyceride transfer protein (MTP), do not produce apolipoprotein B–containing lipoproteins. It was hypothesized that small molecule inhibitors of MTP would prevent the assembly and secretion of these atherogenic lipoproteins. To test this hypothesis, two compounds identified in a high-throughput screen for MTP inhibitors were used to direct the synthesis of a highly potent MTP inhibitor. This molecule (compound **9**) inhibited the production of lipoprotein particles in rodent models and normalized plasma lipoprotein levels in Watanabe-heritable hyperlipidemic (WHHL) rabbits, which are a model for human homozygous familial hypercholesterolemia. These results suggest that compound **9**, or derivatives thereof, has potential applications for the therapeutic lowering of atherogenic lipoprotein levels in humans.

Apolipoprotein B (apoB)–containing lipoproteins [chylomicrons, very low density lipoproteins (VLDL) and their respective metabolic products, chylomicron remnants, and low density lipoproteins (LDL)] promote coronary artery atherosclerosis, which is a leading cause of death in industrialized nations. MTP is a heterodimeric lipid transfer protein consisting of protein disulfide isomerase and a unique 97-kD subunit that is localized in the endoplasmic reticulum of hepatocytes and enterocytes (*1–3*). Defects in MTP cause abetalipoproteinemia (*3–5*), a disorder in which the production of VLDL and chylomicrons is disrupted. Patients with abetalipoproteinemia have plasma cholesterol levels of ~40 mg/dl and plasma triglyceride levels of <10 mg/dl (*6*), whereas normal adults have levels of 180 to 220 and 100 to 150 mg/dl, respectively. These findings suggest that inhibitors of MTP might be therapeutically useful for inhibiting the production of VLDL and chylomicrons, thereby reducing the levels of atherogenic lipoprotein particles.

Abetalipoproteinemia is an extreme example of MTP inhibition and would not be the intended clinical end point for a drug. A related genetic disease, hypobetalipoproteinemia, is caused by mutations in apoB (*6, 7*). Heterozygous people with this disease have half the normal levels of apoB-containing lipoproteins and lack the clinical signs and symptoms of patients with abetalipoproteinemia. Family studies have shown that individuals with hypobetalipoproteinemia have a prolonged life span (*8*).

We performed a high-throughput screen of a large chemical library to identify inhibitors of MTP-mediated triglyceride transfer. This resulted in the discovery of BMS-200150 (compound **1** in Fig. 1), which inhibits the MTP-mediated transport of triglycerides between membranes in vitro (*9*) and inhibits the secretion of apoB-containing lipoproteins from HepG2 cells, a human liver-derived cell line. However, the compound was not active in animal models. Compound **2**, an analog of BMS-200150 with an extended alkyl linker, was of comparable potency in both the lipid transfer and HepG2 apoB secretion assays (Fig. 1 and Table 1). A fluorenyl amide (compound **3**), which is a much less potent inhibitor, was also identified in the high-throughput screening. Subsequently, nitrogen substitution of **3** with a short-chain alkyl group (for example, **4**) was

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found to improve the potency of this series, making it comparable with the series of **1** and **2**.

On the basis of the structural relationships between **1**, **2,** and **4**, it was proposed that **4** binds to MTP at a subdomain of the binding site for **1** and **2**. To test this proposal, we prepared "hybrid" analogs **5** and **6** (Fig. 1). With **5**, the overlap between the fluorenyl amide of **4** and the isoindolone of **1** was explored, resulting in a significant loss in inhibitory potency (Table 1). With **6**, the overlap between the fluorenyl amide of **4** and the diphenylmethyl substituent of **2** was explored, resulting in a dramatic improvement (>100-fold) in potency in both the lipid transfer and HepG2 apoB secretion assays.

Hybrid **6** was tested for its ability to inhibit lipoprotein secretion in fasted rats. This assay was based on the ability of intravenously injected Triton WR1339 to prevent the catabolism of triglyceride-rich lipoproteins (*10*). Thus, after the administration of Triton, there was a linear increase in plasma triglyceride levels, which reflects the triglyceride secretion rate from the liver and intestine. The inhibition of hepatic lipoprotein production can be measured with fasted animals in which the liver is the primary source of plasma triglycerides, whereas in fed rats, there is substantial lipoprotein production in the intestine. In contrast to the earlier analogs, hybrid **6** was a potent and dose-dependent inhibitor of triglyceride secretion in vivo. The corresponding N-CH_2CF_3 analog, **7**, resulted in a dramatic improvement in oral potency.

A modification of the isoindolone portion of **7** indicated that a benzamide (inhibitor **8**) was a suitable replacement for the isoindolone ring. An extensive optimization of this substituent was performed by an automated organic synthesis (*11*), which resulted in the identification of the 4′-CF_3-biphenyl carboxamide, **9**. This MTP inhibitor possesses subnanomolar potency in both the lipid transfer and HepG2 apoB secretion assays and inhibits lipoprotein secretion in fasted rats (Table 1). The intravenous and oral half-maximal effective dose (ED_{50}) values were similar, suggesting that the compound was well-absorbed and bioavailable to the liver. Compound **9** was equally effective in inhibiting acute lipoprotein secretion in fasted or fed rats (Fig. 2), indicating that it inhibits both hepatic and intestinal lipoprotein secretion.

The effect of compound **9** on plasma lipid levels was tested in hamsters which, unlike other rodents, transport a substantial proportion of their cholesterol on LDL. Hamsters that were fed a standard, low-fat, high-carbohydrate diet were treated once daily with doses of compound **9** of 1, 3, or 6 mg per kilogram of body weight (mg/kg) for 7 days. After the final drug treatment, the animals were fasted for 18 hours, and their plasma lipid and lipoprotein levels were then evaluated (Fig. 3). A dose-dependent decrease in the total plasma cholesterol was observed, with an ED_{50} value for cholesterol lowering of 2.4 mg/kg. The triglyceride, VLDL and LDL cholesterol, and high density lipoprotein (HDL) cholesterol levels all decreased in parallel with the decrease in total plasma cholesterol. The HDL decrease was unlikely to be due to a direct effect on HDL production because, in HepG2 cells, the ED_{50} for the inhibition of secretion of apolipoprotein AI (apoAI; the major structural protein of HDL) by compound **9** is over 8000-fold higher than that for the inhibition of secretion of apoB (Table 1). At the 6 mg/kg dose, the total plasma cholesterol and triglyceride levels were decreased by 90 and 49%, respectively, in comparison to the control animals. When animals were treated with compound **9** for up to 3 weeks, there were similar findings. There were no clinically relevant differences between vehicle- and drug-treated animals in body weight, plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), or any other plasma chemistry variables that were tested (*12*).

Hamsters that were treated with compound **9** did not have steatorrhea, as indicated by the absence of fat (<0.01 g per 24 hours) in their stools. After killing these hamsters, we noted visual and biochemical evidence of fat accumulation in enterocytes. The liver triglyceride content of animals that were treated with compound **9** reached a plateau after 1 week of treatment, never exceeded 25 mg per gram of tissue (*13*), and returned to control levels 48 hours after the termination of a 3-week treatment regimen. In comparison, fatty livers in humans contain up to 35% triglyceride by weight (*14*). Liver weight in the hamsters (as percent body weight) showed minimal change with treatment (*15*). Plasma transaminase levels did not rise sig-

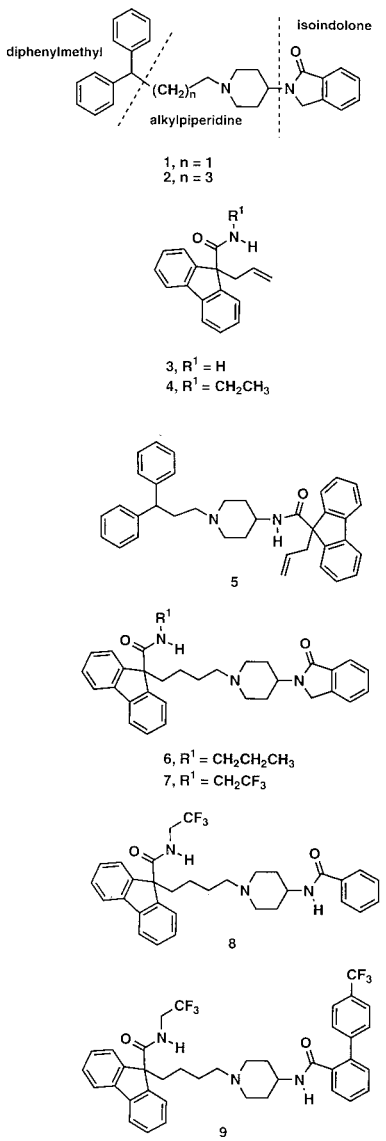

Fig. 1. Structures of MTP inhibitors.

Table 1. Comparative activity of compounds as MTP inhibitors measured in vitro, in cell culture, and in vivo. The in vitro and cell culture assays were performed as described in (*9*). The fasted rat assay was performed as described in Fig. 2 and (*10*). For intravenous (iv) dosing, the compound was directly incorporated into Triton WR1339. Human MTP was produced in Sf9 insect cells with the baculovirus expression system (*4*). IC_{50}, concentration that produces half-maximal inhibition; ED_{50}, dose that produces a half-maximal effect; IA@2, IA@15, and IA@100, inactive at doses 2, 15, and 100 mg/kg, respectively; ND, not done.

Compound	IC_{50} values of triglyceride transfer of human MTP in vitro (nM)	ED_{50} values of secretion of apoB and apoAI from HepG2 cells (nM)		ED_{50} values of secretion of triglycerides in rats (mg/kg)	
		apoB	apoAI	iv	Oral
1	2,200	1,800	>30,000	IA@2	IA@15
2	5,800	1,100	11,000	ND	ND
3	36,000	17,000	>33,000	ND	ND
4	2,200	4,500	>33,000	ND	IA@100
5	25,300	2,600	4,600	ND	ND
6	12	19	>3,300	0.28	15
7	36	3	>3,300	1.2	1.9
8	23	8.1	68,000	ND	0.7
9	0.5	0.8	6,500	0.15	0.19

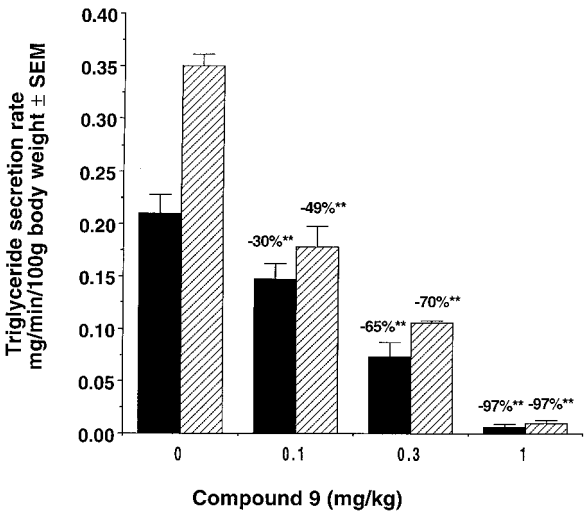

Fig. 2. Inhibition by compound **9** of triglyceride secretion in fasted and fed rats. Sprague-Dawley rats (~200 g each, four per treatment group) were adapted to a reversed diurnal light cycle for 2 weeks. Before the experiment, the rats either were fasted (solid bars) or had free access to food (hatched bars) for 18 hours. The animals were orally dosed with compound **9** in a vehicle (10% M-pyrol, 80% water, 5% cremophore, and 5% ethanol) 1 hour before receiving an intravenous injection of Triton WR1339 (250 mg/kg). The triglyceride secretion rate was determined by calculating the amount of triglyceride that was accumulated in plasma during the 2.5 hours after the Triton injection. The standard assay was linear for at least 5 hours after the Triton injection. Plasma triglyceride levels were determined with a Roche Cobas blood chemistry autoanalyzer (a negative percentage indicates a decrease). The animals were cared for in accordance with institutional guidelines. Error bars indicate SEM. **, significantly different from vehicle-treated animals at $P < 0.005$.

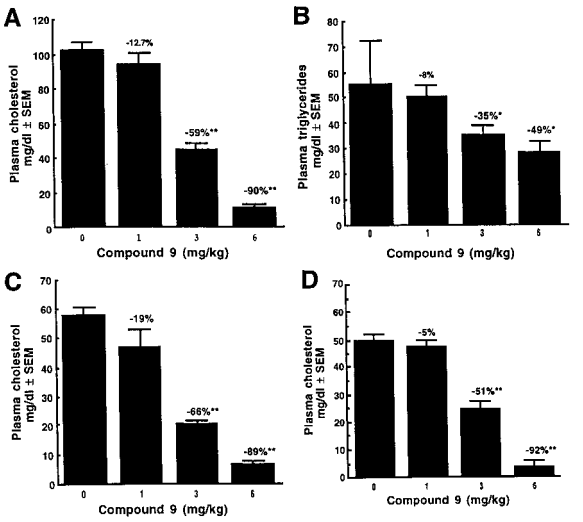

Fig. 3. Effect of compound **9** on (**A**) total cholesterol, (**B**) triglycerides, (**C**) VLDL and LDL cholesterol, and (**D**) HDL cholesterol levels in hamsters (a negative percentage indicates a decrease). Male Golden Syrian hamsters (~140 g each, four per treatment group) were adapted to and maintained on a reverse diurnal light cycle. They were dosed orally once a day with compound **9** in a vehicle (10% M-pyrol, 80% water, 5% cremophore, and 5% ethanol) and were allowed free access to a standard hamster diet (Purina 5001, which contains 0.02% cholesterol and 4.5% triglyceride). After 7 days of drug treatment, hamsters were fasted for 18 hours, after which plasma lipid levels and chemistries were determined with a Roche Cobas blood chemistry autoanalyzer. Hamster lipoprotein fractions were quantitated after the precipitation of apoB-containing lipoproteins with phosphotungstate and magnesium chloride. Drug effects were calculated in relation to the vehicle control group. Error bars indicate SEM.*, $P < 0.05$; **, $P < 0.005$.

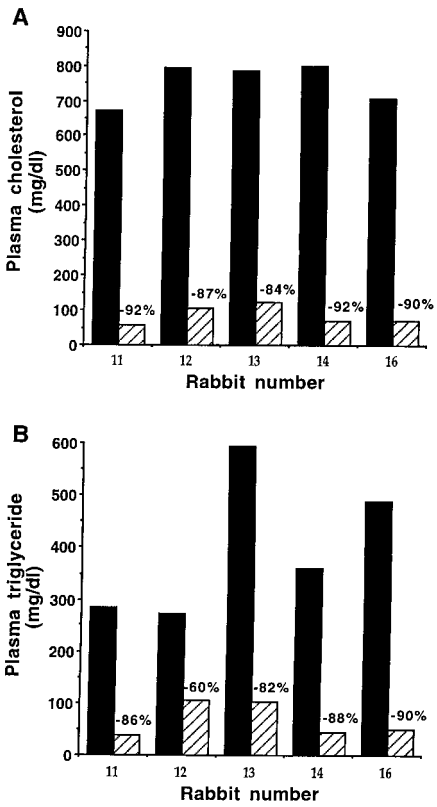

Fig. 4. Effect of compound **9** on plasma lipid levels in WHHL rabbits (a negative percentage indicates a decrease). Five rabbits were treated orally for 14 days with compound **9** (10 mg/kg). Plasma (**A**) total cholesterol and (**B**) triglyceride levels were measured 18 hours after the last dose. Prebleed, solid bars; 14-day treatment, hatched bars.

nificantly over a 3-week treatment period, and triglyceride accumulation was reversible, which suggest that the modest increase in hepatic lipid had no adverse effects in the hamster model.

We also investigated the effect of a 2-week treatment with compound **9** in homozygous Watanabe-heritable hyperlipidemic (WHHL) rabbits whose hepatic LDL receptor activity is <5% that of normal rabbits, resulting in dramatically elevated levels of apoB-containing lipoproteins. WHHL rabbits are a model for human homozygous familial hypercholesterolemia (FH) (*16*). The elevated plasma cholesterol levels in FH patients (600 to 1200 mg/dl) cannot be normalized with current therapies, which typically lower LDL cholesterol levels by a maximum of 30%. The ED_{50} value for cholesterol lowering in WHHL rabbits that were treated with compound **9** was 1.9 mg/kg. Triglyceride levels were lowered in parallel. At a dose of 10 mg/kg of **9**, the plasma levels of atherogenic, apoB-containing, lipoprotein particles were essentially normalized (Fig. 4) with no alteration in plasma AST or ALT (*17*).

In the search for an understanding of lipoprotein metabolism and a means to treat atherosclerosis, much effort has been focused on hyperlipidemic states. This focus has resulted in the identification of genetic defects that produce loss of functions that cause hyperlipidemia. Our results highlight the importance of understanding hypolipidemic states. Identifying a loss-of-function gene defect for a genetic disorder that is the physiological opposite of the disease that one is attempting to treat allows one to identify a target that is theoretically amenable to pharmacological intervention (that is, a normal protein for which an inhibitor mimicking the effect of the mutation will produce the desired therapeutic outcome). With current technologies, it is easier to identify a small molecule inhibitor of a normal activity than to augment a deficient activity. This strategy yielded compound **9**, which has the potential to be an effective antiatherogenic agent and is now in clinical trials. Similar drug discovery strategies may be applicable to other diseases.

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13. The milligrams of triglyceride measured per gram of wet weight liver for treatments with vehicle and 1, 3, and 6 mg/kg doses of compound **9** were as follows: 9.1 ± 0.6 (mean ± SEM), 11 ± 0.3, 18 ± 1.8, and 24 ± 0.4, respectively, at 1 week; 8 ± 0.4, 11 ± 1.1, 12 ± 0.8, and 19 ± 3.4 at 2 weeks; and 9 ± 0.5, 11 ± 1.5, 23 ± 3.5, and 19 ± 1.0 at 3 weeks.
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18 June 1998; accepted 23 September 1998

Genome Sequence of an Obligate Intracellular Pathogen of Humans: *Chlamydia trachomatis*

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Analysis of the 1,042,519–base pair *Chlamydia trachomatis* genome revealed unexpected features related to the complex biology of chlamydiae. Although chlamydiae lack many biosynthetic capabilities, they retain functions for performing key steps and interconversions of metabolites obtained from their mammalian host cells. Numerous potential virulence-associated proteins also were characterized. Several eukaryotic chromatin-associated domain proteins were identified, suggesting a eukaryotic-like mechanism for chlamydial nucleoid condensation and decondensation. The phylogenetic mosaic of chlamydial genes, including a large number of genes with phylogenetic origins from eukaryotes, implies a complex evolution for adaptation to obligate intracellular parasitism.

Chlamydia are bacterial pathogens whose representatives are widely distributed in nature, and *C. trachomatis* causes several human diseases of medical significance. Ocular infection leads to trachoma, a leading cause of preventable blindness. Of all infectious diseases reported to U.S. state health departments and the U.S. Centers for Disease Control and Prevention, chlamydial genital tract infections are the most common, and infection of the genital tract often results in pelvic inflammatory disease, ectopic pregnancy, chronic pelvic pain, epididymitis, and infant pneumoniae (*1*). *Chlamydia trachomatis* genital tract infections may also significantly increase the risk for HIV infection (*2*).

Chlamydiae are deeply separated from other eubacteria and represent one of the kingdom-level branches of the phylogenetic tree (*3*). After invasion of eukaryotic cells, chlamydiae grow within an intracellular vacuole, called an inclusion, that does not fuse with lysosomes. Microbiologically, *Chlamydia* are characterized by a developmental cycle involving a metabolically inactive infectious developmental form called the elementary body (EB) that, after entry into the target host cell, differentiates into a metabolically active developmental form called the reticulate body (RB). At approximately 20 hours post infection and after multiple divisions by binary fission, the RB differentiates into the EB developmental stage and infectious EBs are released to initiate new rounds of infection. Chlamydial physiology, structure, developmental biology, and genetics are poorly understood. The limited and obligate intracellular growth of chlamydiae and the lack of any direct or indirect genetic methods for their study has restricted the development of biological and molecular understanding of these unusual organisms (*4*).

The sequenced chlamydial genome consists of a 1,042,519–base pair chromosome (58.7% A+T) and a 7493–base pair plasmid (sequence and annotation available at http://chlamydia-www.berkeley.edu:4231 and GenBank under accession number AE001273). Analysis of the chlamydial genome resulted in the identification of 894 likely protein-coding genes (*5*). Similarity searching permitted the inferred functional assignment of 604 (68%) encoded proteins, and 35 (4%) were similar to hypothetical proteins deposited for other bacteria. The remaining 255 (28%) predicted proteins were not similar to other sequences deposited in GenBank. Clustering by sequence similarity showed that 256 chlamydial proteins (29%) belong to 58 families of similar genes within the genome (paralogs), a fraction similar to other bacteria with relatively small genomes such as the mycoplasmas and *Haemophilus influenzae* (*6*). A list of the results of analysis of the predicted chlamydial proteins classified in accord with the functional systems in this bacterium and a linear map of genes are available (*5*). The most prominent findings are presented below.

Counterparts of enzymes characterized in other bacteria (orthologs) were identified in *C. trachomatis* to account for the minimal requirements for DNA replication, repair, transcription, and translation. DNA repair and recombination systems were extensively represented in the chlamydial genome, indicating that chlamydiae have considerable recombination capabilities. There are also two predicted DNA he-

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Science **282**, 751 (1998);
DOI: 10.1126/science.282.5389.751