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Microsomal Triglyceride Transfer Protein Inhibitors: Discovery and Synthesis of Alkyl Phosphonates as Potent MTP Inhibitors and Cholesterol Lowering Agents

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Abstract—A series of newly synthesized phosphonate esters were evaluated for their effects on microsomal triglyceride transfer protein activity (MTP). The most potent compounds were evaluated for their ability to inhibit lipoprotein secretion in HepG2 cells and to affect VLDL secretion in rats. These inhibitors were also found to lower serum cholesterol levels in a hamster model upon oral dosing.

For more than 20 years there has been a great deal of interest by the pharmaceutical industry in developing agents which alter circulating lipid levels.[1,2] The HMG-CoA Reductase (HMG-R) inhibitors,[3,4] by nature of their ability to lower primarily serum cholesterol levels, have been shown to reduce mortality. These clinical findings have led to a search for alternate lipid lowering therapies which impact triglyceride levels as well. Of particular interest are agents that target inhibition of the assembly of apolipoprotein B (apoB)—containing lipoproteins. Inhibition of the assembly process should reduce both the production of intestinal chylomicrons and the secretion of hepatic very low density lipoproteins (VLDL). In addition, lowering of the respective metabolic products, chylomicron remnants and low density lipoproteins (LDL), should also be expected. High levels of these products are known indicators for an increased risk of arterial atherosclerosis. Hence, reduction in these known risk factors will have a beneficial effect on the potential for coronary heart disease.

Moreover, patients with abetalipoproteinemia, a disease caused by defects in microsomal triglyceride transfer protein (MTP), are known to have low levels of plasma lipids (total cholesterol: 40 mg/dL; triglycerides: <10 mg/dL) and are characterized by a near absence of apoB-containing lipoproteins.[5] It has also been shown in vitro that MTP catalyzes the transport of lipid molecules between phospholipid membranes, suggesting that MTP is involved in the synthesis of nascent lipoprotein particles within the lumen and endoplasmic reticulum.[6] The findings that patients with abetalipoproteinemia have low levels of cholesterol and triglycerides and that MTP catalyzes lipid transport suggests that interruption of the assembly of apoB-containing lipoproteins by inhibition of MTP would lead to a reduction in circulating atherogenic lipid levels.[7]

We recently disclosed a series of MTP inhibitors (**4**) which normalized plasma lipoprotein levels in Watanabe-heritable hyperlipidemic (WHHL) rabbits.[8] These inhibitors were developed by hybridization of leads **1** and **2**, identified by our high-throughput screening assay.

In addition to leads **1** and **2**, phosphonate **3** was also uncovered in the high-throughput screen. This

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phosphonate contained several similarities to inhibitors **4**. Specifically, the left hand side of both molecules contain a hydrophobic aromatic domain and the right hand portion contains both a hydrogen bond acceptor as well as a lipophilic substituent. In the case of **3** and **4**, the hydrogen bond acceptors are the phosphonate P=O and the benzamide C=O respectively. Additionally, the phosphonate butyl ester substituents contained in **3** could loosely mimic the remaining phenyl fragment in **4** (Fig. 1). In both structures, a hydrocarbon spacer bridges these key features.

This paper highlights the in vitro structure–activity relationships around inhibitor **3** and the development of hybrid inhibitors derived from merging the left hand fragment of **1** to the right hand fragment of **3**. The latter compounds demonstrate efficacy in reducing lipoprotein secretion in both cell-based and animal models. Select analogues showed cholesterol-lowering activity in a hamster model.

Replacement of the Left Hand Aryl Fragment of 3

The initial focus to optimize the phosphonate screening lead **3** was through coupling of commercially available aromatic nucleophiles under basic conditions (K_2CO_3, DMF) with bromide **5** (Scheme 1). Simple filtration and solvent removal gave essentially pure materials. Further purification could be achieved by flash column chromatography. The products obtained were intended to acquire information on the optimal tether length between the phosphonate moiety and the hydrophobic domain as well as to search for a more optimal hydrophobic fragment.

While over 60 compounds were prepared, only a brief survey of these is listed in Table 1 thus providing a representative SAR for these compounds. Inhibition of MTP was assayed by measurement of triglyceride transfer between donor and acceptor unilamellar vesicles.[9] Decreasing the size of the hydrophobic domain in **3** resulted in weaker activity (inhibitors **7**, **8**, **10**). Branching the aromatic in group in **3** to a diphenylheterocyle (inhibitors **11**, **12**) causes a modest loss of activity. Altering the linker between the aromatic fragment and the phosphonate group (**13** and **14**) gave no improvement in activity. Inhibitors (**7**–**17**) were either as active as the parent (**3**) or demonstrated weaker MTP inhibitory activity. With the lack of any significant improvement in inhibitory activity and limited structural activity relationship between compounds **3** and **7**–**17** an alternate approach to generate potent inhibitors of MTP was attempted.

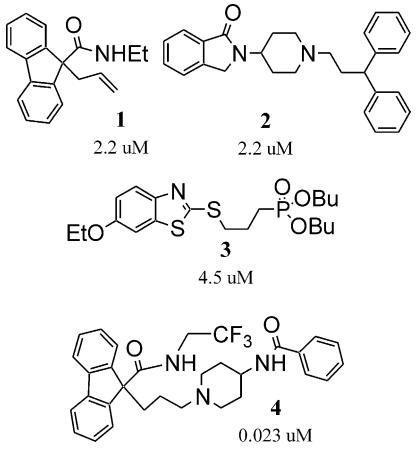
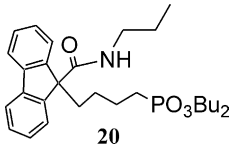


Figure 1. IC_{50} values.

Scheme 1.

Recognizing the loose similarity between the hydrophobic domains of **1** (fluorenyl moiety) and **3** (benzthiazole) and on the basis of the high degree of potency of **4**, a hybrid-inhibitor was proposed (**20**). The hybrid-structure would afford all the binding interactions in **3** and additionally include a secondary amide group. The amide functionality would have a potential to act as a hydrogen bond acceptor (C=O) as well as position a lipophilic appendage as seen in **4**.

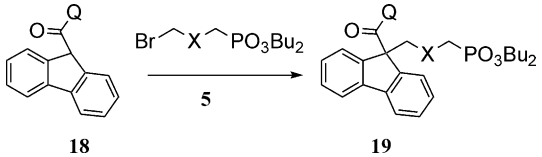


Inhibitors of this series were prepared according to the chemistry methods outlined in Scheme 2. Specifically, the carboxylic acid **18** (Q=OH) was treated with 2 equivalents of butyllithium and then allowed to react

Table 1. Inhibition of MTP by aromatic phosphonates[a,b]

Compd	Nu	X	IC_{50}, μM
3	6-Ethoxy-2-mercaptobenzothiazole	CH_2	4.5±1.2
7	Phenol	CH_2	48±4.3
8	2-Mercapto-thiophene	CH_2	>100
9	α-Napthol	CH_2	30±4.2
10	4-Mercapto-pyidine	CH_2	27±3.2
11	4,5-Diphenyl-2-mercapto-oxazole	CH_2	6±2
12	4,5-Diphenyl-2-mercapto-imidazole	CH_2	>30
13	6-Ethoxy-2-mercaptobenzothiazole	$(CH_2)_2$	12±4
14	6-Ethoxy-2-mercaptobenzothiazole	$(CH_2)_3$	6±1.3
15	6-Ethoxy-2-mercaptobenzoxazole	CH_2	5±2.2
16	6-Ethoxy-2-mercaptobenzimidazole	CH_2	10±3.3
17	*N*-Methyl-6-ethoxy-2mercapto-benzimidazole	CH_2	6±0.5

[a]All IC_{50} values listed in tables are the mean of three experiments in the inhibition of lipid transport between vesicles.
[b]For a detailed description of the lipid transport assay conditions see ref 9.



Scheme 2. (1) THF, 2 equiv of *n*-BuLi, −78 °C; then **5** from −78 °C to rt; (2) $(COCl)_2$, DMF (catalyst), dichloromethane; then amine, TEA.

with bromide **5**. Simple conversion of carboxylate to an amide was achieved with $(COCl)_2$ and a primary amine to give compounds **20**–**30** (Q=CONHR).

Exchange of the 6-ethoxy-2-mercaptobenzthiazole group in **3** with the fluorenyl carboxamide found in **1** produced a dramatic 300-fold increase in inhibitory activity (compound **20**). Additionally, as seen in Table 2, modest changes in the amide appendage gave rise to significant changes in activity in the lipid transport assay. For instance, the amide **20** is much more potent than the ester **25**. The carboxylate (not shown) was inactive. As a general trend, simple alkyls were more active than alkylaryls (cf. **20** vs **21**); however activity could be recovered in the arylalkyl series by additional substitution from the aromatic nucleus (**26**). Attempts to increase water solubility with either morpholine or pyridyl substituents provided compounds that were markedly less active than **20**. The effect of the spacer between the fluorenyl and the phosphonate group was evaluated. The maximal effect was obtained when the spacer was butylene; elongation of the tether decreased activity (**28**–**30**).

Effect of Phosphonate Ester Substituents

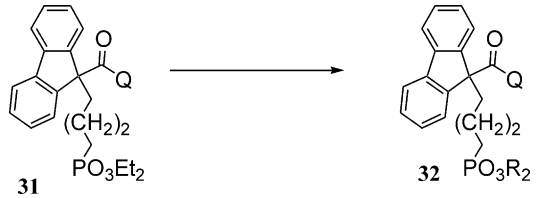
With the discovery that the fluorenyl-phosphonate hybrids possessed excellent potency, variation of the butyl group of the phosphonate moiety was examined. A concise synthetic sequence (Scheme 3) was followed where the phosphonate ethyl ester (**31**) was treated with TMSBr followed by oxalyl chloride to give the phosphonyl chloride. The chloride rapidly reacted with an alcohol and triethylamine to generate compounds **33**–**44**.

The data provided in Table 3 illustrate several key points. Evaluation of the various ester substituents reveals the importance of ester length to binding affinity. For example, the longer butyl ester imparts enhanced activity when compared to the shorter ethyl derivative. Interestingly, the effect of branching provides mixed results. The iso-butyl ester was less active than the *n*-butyl ester. Increasing branching further to a neo-pentyl group improved activity. Introduction of pyridyl esters, substituents that provided increased water solubility, led to the formation of **44**. This 6-methyl-2-pyridyl ester phosphonate was found to be a particularly potent analogue ($IC_{50}=0.011\,\mu M$).

Table 2. Inhibition of MTP by fluorenyl phosphonates: effect of the fuorenyl amide substituent

Compd	Q	X	IC_{50}, μM
20	$NHCH_2CH_2CH_3$	$(CH_2)_2$	0.014±0.002
21	$NHCH_2Ph$	$(CH_2)_2$	0.25±0.010
22	$NHCH_2Ph$-4-NH_2	$(CH_2)_2$	0.89±0.014
23	$NHCH_2(CH_2)_2CH_3$	$(CH_2)_2$	0.40±0.21
24	$NHCH_2$-2-Pyridyl	$(CH_2)_2$	0.47±0.14
25	OCH_3	$(CH_2)_2$	0.45±0.16
26	$NHCH_2CH_2Ph$-4-Ome	$(CH_2)_2$	0.021±0.08
27	$NHCH_2CH_2$-N-morpholine	$(CH_2)_2$	0.50±0.10
28	$NHCH_2CF_3$	$(CH_2)_4$	0.045±0.07
29	$NHCH_2CF_3$	$(CH_2)_3$	0.065±0.009
30	$NHCH_2CF_3$	$(CH_2)_2$	0.022±0.010

IC_{50} values for the inhibition of lipid transport between vessicles.



Scheme 3. (1) (a) TMSBr; (b) $(COCl)_2$, DMF; (c) ROH, TEA.

Inhibition of HepG2 Cell Lipoprotein Secretion

As can be seen in Table 4, compound **20** is a potent inhibitor of apoB secretion in HepG2 cells[9] ($EC_{50}=0.1\,\mu M$) and was 50-fold more potent than the original screening lead **3**. Activity was maintained when the propyl amide was converted into the benzyl or substituted benzyl substituents (**21** and **26**). Interestingly, the trifluoroethyl analogue **30** was very potent ($IC_{50}=0.007\,\mu M$).[10] In the case of **30**, comparison of the ED_{50}'s for the inhibition of apoB-containing and apoAI-containing lipoproteins revealed a >470-fold degree of selectivity. When the selectivity of **30** is compared to initial screening lead **3** a 140-fold change was

Table 3. Inhibition of MTP by fluorenyl phosphonates: effect of the phosphonate alkyl substituent

Compd	Q	R	IC_{50}, μM
20	NH-*n*-Pr	Bu	0.014±0.002
31	NH-*n*-Pr	Et	0.135±0.071
30	$NHCH_2CF_3$	Bu	0.022±0.006
33	$NHCH_2CF_3$	Et	0.145±0.062
34	$NHCH_2CF_3$	n-Pr	0.090±0.212
35	$NHCH_2CF_3$	*i*-Pr	0.280±0.071
36	$NHCH_2CF_3$	*i*-Bu	0.055±0.003
37	$NHCH_2CF_3$	Neo-pentyl	0.033±0.012
38	$NHCH_2CF_3$	CH_2-3-pyridyl	0.024±0.003
39	$NHCH_2CF_3$	CH_2-4-pyridyl	0.085±0.007
40	$NHCH_2CF_3$	CH_2-2-pyridyl	0.027±0.003
41	$NHCH_2CF_3$	CH_2CH_2-2-pyridyl	0.040±0.010
42	$NHCH_2CF_3$	$CH_2(CH_2)_2$-2-pyridyl	0.025±0.001
43	$NHCH_2CF_3$	$(CH_2)_3$-2-(6-Me-pyridyl)	0.045±0.014
44	$NHCH_2CF_3$	CH_2-2-(6-Me-pyridyl)	0.011±0.002

IC_{50} values for the inhibition of lipid transport between vesicles.

Table 4. Comparative activity of phosphonates as inhibitors of MTP in HepG2 cells

Compd	IC_{50}, μM[a]	EC_{50} values of secretion of ApoB and ApoA1 from HepG2 cells (μM)[b]	
		ApoB	ApoA1
3	4.5	3.9±1.2	13.2±4.2
20	0.014	0.1±0.04	8.0±2.0
21	0.25	0.058±0.012	>1.0
26	0.021	0.023±0.008	>0.33
30	0.022	0.007±0.003	>3.3
4	0.023	00.08±0.005	>3.3

[a]IC_{50} values for the inhibition of lipid transport between vesicles.
[b]For a detailed description of the conditions of the EC_{50} apolipoprotein secretion assay see ref 9.

realized. The effect on selectivity is consistent with inhibition of MTP: where assembly of the ApoB lipoproteins (VLDL) should be inhibited while little effect would be expected on the secretion of apoA1-containing lipoproteins.

Inhibition of Hepatic Lipoprotein Secretion

Compounds **20**, **26** and **30** were tested for their ability to inhibit lipoprotein production in vivo. The fasted triton rat assay[11] provided a pharmocodynamic model of MTP inhibition where the ability of a compound to inhibit secretion of triglyceride rich VLDL particles could be measured. Animals were dosed with inhibitor either orally or intravenously followed after 1 h with an intravenous injection of Triton WR-1339. The triglyceride secretion rate was determined by calculating the amount of triglyceride that accumulated after 2.5 h after the triton injection. Compound **20** had an ED_{30} of 15 mg/kg (intravenous) and was inactive at 50 mg/kg orally. Hybrid **30** had an ED_{30} of 4 and 8 mg/kg on iv and oral dosing respectively. The more potent N–CH_2CF_3 analogue (in HepG2 cells) demonstrated a beneficial effect upon both oral and intravenous dosing. The potent activity seen in HepG2 cells with **26** ($EC_{50} = 0.023\ \mu M$) was only partially translated in the rat model (−15% @ 25 mg/kg).

Cholesterol Lowering in Hamsters

In previous studies we have shown that sub-chronic oral administration of MTP inhibitors in normal chow-fed hamsters could effect significant changes in total plasma cholesterol. Thus, the hamster served as a reasonable pharmacodynamic model in which to evaluate these compounds. Inhibitors **30**, **40**, **42**, and **44** were administered orally (qd) for 3 days at doses ranging between 15 and 100 mg/kg. Compound **30** was weakly active showing only a modest response (−15%) at @ 100 mg/kg. In contrast, the 2-pyridyl substituted compounds **42** and **44** were more efficacious in this model. Compound **42** (−15% at 15 mg/kg/day) demonstrated greater activity than **30**. The most active compound investigated was **44**, where 35% cholesterol lowering was observed at a dose of 15 mg/kg/day over 3 days.

Our studies have demonstrated MTP inhibitors such as **44** exhibit potent MTP inhibition both in vitro and in vivo. These inhibitors are a new class of MTP inhibitors and cholesterol lowering agents. They compare favorably with the forerunner **4** in in vitro activity and in cell culture. These findings were also translated to the hamster cholesterol lowering model where compound **44** was found to be similar in activity to compound **4**.

References and Notes

1. Rosamond, W. D.; Chambless, L. E.; Folsom, A. R.; Cooper, L. S.; Conwill, D. E.; Clegg, L.; Wang, C.-H.; Heiss, G. *N. Engl. J. Med.* **1998**, *339*, 861.
2. Murray, C. J. L.; Lopez, A. D. *Lancet* **1997**, *349*, 1269.
3. Lablanche, J.-M. *Curr. Med. Res. Opin.* **2001**, *16*, 285.
4. Chin-Dusting, Jaye P. F.; Shaw, James A. *Expert Opin. Pharmacother.* **2001**, *2*, 419.
5. Kane, J. P.; Havel, R. J. In *Metabolic and Molecular Basis of Inherited Disease*, Scriver, C. R.; Beaudet, A. L.; Sly, W. S.; Valle, D., Eds.; McGraw-Hill: New York, 1995; pp 1853–1885.
6. Wetterau, J. R.; Zilversmit, D. B. *Biochem. Biophys. Acta* **1986**, *875*, 610.
7. Wetterau, J. R.; Gordon, D. *A. Adv. Vasc. Biol.* **1999**, *5*, 187.
8. Wetterau, J. R.; Gregg, R. E.; Harrit, T. W.; Arbeeny, C.; Cap, M.; Connolly, F.; Chu, C.-H.; George, R. J.; Gordon, D. A.; Jamil, H.; Jolibois, K. G.; Kunselman, L. K.; Lan, S.-J.; Maccagnan, T. J.; Ricci, B.; Yan, M.; Young, D.; Chen, Y.; Fryszman, O. M.; Logan, J. V. H.; Musial, C. L.; Poss, M. A.; Robl, J. A.; Simpkins, L. M.; Slusarchyk, W. A.; Sulsky, R.; Taunk, P.; Magnin, D. R.; Tino, J. A.; Lawrence, R. M.; Dickson, J. K., Jr.; Biller, S. A. *Science* **1998**, *282*, 751.
9. Jamil, H.; Gordon, D. A.; Eustice, D. C.; Brooks, C. M.; Dickson, J. K., Jr.; Chen, Y.; Ricci, B.; Chu, C.-H.; Harrity, T. W. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11991.
10. Koksh, B.; Sewald, N.; Hofmann, H. J.; Burger, K.; Jakubke, H. D. *Journal of Peptide Science* **1997**, *3*, 157.
11. Borensztajn, R. M.; Rone, M.; Kotlar, T. *Biochem. J.* **1976**, *156*, 539.