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Discovery of benzothiazole derivatives as efficacious and enterocyte-specific MTP inhibitors

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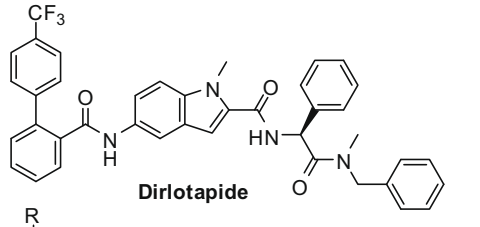
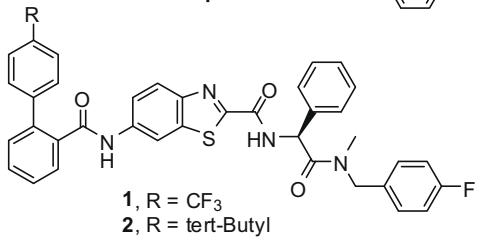
ABSTRACT

A series of triamide derivatives bearing a benzothiazole core is shown to be potent microsomal triglyceride transfer protein (MTP) inhibitors. In order to minimize liver toxicity, these compounds have been optimized to have activity only in the enterocytes and have limited systemic bioavailability. Upon oral administration, selected analogs within this series have been further demonstrated to reduce food intake along with body weight and thereby improve glucose homeostasis and insulin sensitivity in a 28-day mice diet-induced obesity (DIO) model.

In recent years, the microsomal triglyceride transfer protein (MTP) has been the subject of extensive research in many laboratories because of its potential benefits in treating various metabolic diseases. With its distribution in both the intestinal and liver tissues, MTP has been shown to play a crucial role in the assembly of triglyceride-rich chylomicrons in the enterocytes and very low density lipoproteins (VLDL) in the hepatocytes.[1] Inhibition of MTP by small molecules, therefore, should lead to a reduction in plasma triglycerides and cholesterol levels. Early research efforts in this field have resulted in a number of clinical candidates such as CP-346086[2] and BMS-201038.[3] These compounds are systemically bioavailable and have been shown to inhibit MTP in both the enterocytes and in the liver. However, extensive inhibition of MTP in the liver could lead to significant safety issues such as fatty liver disease.[4] One potential way of minimizing this harmful side effect is to selectively inhibit MTP in the enterocytes.[5] In theory, a non-systemic and enterocyte-selective MTP inhibitor will cause a disruption of lipid transport in the endoplasmic reticulum and could afford beneficial weight loss through appetite suppression and intestinal fat absorption. Appetite suppression is believed to be the result of an increase in circulating satiety signaling peptides[6] such as PYY and GLP-1 upon fat accumulation in the enterocytes.[7] Dirlotapide is an enterocyte-specific MTP inhibitor that has recently been approved by the FDA as an anti-obesity agent.[8] Herein, we would like to describe the synthesis and MTP activity for a novel series of triamide derivatives based on the benzothiazole template, as illustrated in structures **1** and **2**.





1, R = CF_3
2, R = tert-Butyl

The critical methyl 6-aminobenzo[*d*]thiazole-2-carboxylate derivative that is needed for the synthesis of our MTP inhibitors is prepared according to the steps outlined in Scheme 1. 2-Bromo-4-nitroaniline (**3**) was reacted first with 4,5-dichloro-1,2,3-dithiazol-1-ium chloride (**4**), also referred to as Appel's salt,[9] to form (*Z*)-2-bromo-*N*-(4-chloro-5*H*-1,2,3-dithiazol-5-ylidene)-4-nitroaniline (**5**). This intermediate could be cyclized to the desired benzothiazole intermediate **6** upon heating with CuI in pyridine.[10] 6-Nitrobenzo[*d*]thiazole-2-carbonitrile could then be converted to 6-aminobenzo[*d*]thiazole-2-carboxylate by first reacting with sodium methoxide,[11] followed by standard reduction of the nitro group. The resulting amine **7** could be coupled with 4′-(trifluoromethyl)biphenyl-2-carboxylic acid using the standard protocols described earlier[8] to obtain the MTP inhibitor **1**.

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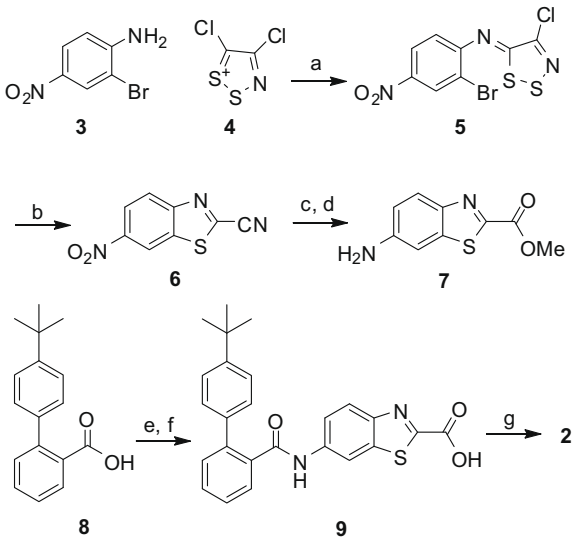


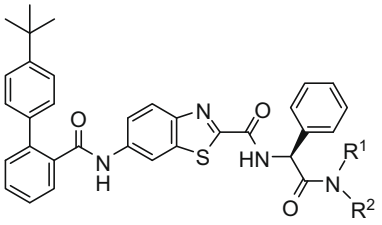
Scheme 1. Reagents and conditions: (a) THF, rt (40% yield); (b) CuI, pyridine, 110 °C (70% yield); (c) NaOMe, MeOH (95% yield); (d) H_2, 1 atm, 10% Pd on C, 1:1 MeOH/EtOAc (>95% yield); (e) oxalyl chloride, CH_2Cl_2, cat. DMF; **7**, CH_2Cl_2, THF, Et_3N; (f) $LiOH{\cdot}H_2O$, MeOH, H_2O, rt (65% yield); (g) HATU, DIEA, (*S*)-2-amino-*N*-(4-fluorobenzyl)-*N*-methyl-2-phenylacetamide hydrochloride, CH_3CN (73% yield).

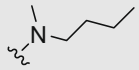
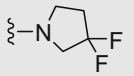
Previously, the 4′-(trifluoromethyl)biphenyl group has been used extensively as one of the key components of MTP inhibitors.[1] However, since our goal is to obtain non-systemic MTP inhibitors, we were interested in incorporating metabolically labile groups at this portion of the molecule. The 4′-(*tert*-butyl)biphenyl group is potentially more metabolically labile than the 4′-(trifluoromethyl)biphenyl group and has been used successively before in this area.[12] The acid **8** that was needed could be conveniently obtained through standard Palladium coupling between 4-*tert*-butylphenylboronic acid and ethyl 2-iodobenzoate, followed by ester hydrolysis. Standard amino acid coupling between **8** and **7**, followed by ester hydrolysis, afforded the acid derivative **9**. Routine amide coupling between **9** (*S*)-2-amino-*N*-(4-fluorobenzyl)-*N*-methyl-2-phenylacetamide hydrochloride afforded the MTP derivative **2**.

In order to assess for MTP inhibitory activity, we employed the HepG2 cellular assay as the primary screen and the enzyme assay as the secondary screen to insure on-target activity. Both assays have been used extensively in this field and have been described in detail previously.[12] In the HepG2 assay, compound **1** had an IC_{50} of 17.3 nM. The *tert*-butyl derivative **2** was more active in the HepG2 cells, with an IC_{50} of 2.7 nM. The MTP activity of compound **2** was further confirmed by the enzyme assay (IC_{50} = 3.0 nM). We have since varied the capping amine portion of derivative **2** and have not observed significant changes in the MTP activity (Table 1, analogs **10–13**). In our series, the (*S*)-configuration at the phenylglycine moiety was more important for activity. Incorporation of the (*R*)-configuration at this position resulted in significant loss of MTP activity. For instance, compound **11**, with an (*S*)-configuration had an IC_{50} of 1.5 nM in the HepG2 assay. The corresponding (*R*)-isomer of **11** was significantly less active, with an IC_{50} of 34.8 nM.

Since modifications in the capping amine portion did not result in substantial changes in MTP activity, we turned our attention to the 4′-(*tert*-butyl)biphenyl group, the carboxamide group at the C6 position of the benzothiazole. Table 2 lists a number of different carboxamide groups that we have looked into. Compound **14**, with an isopropyl group instead of a *tert*-butyl group, was comparable in MTP activity, both in the HepG2 and in the enzyme assay. Incorporating a methyl group or methoxy group at the remaining open

Table 1
Benzothiazole derivatives with varying capping amines



Compound	—NR^1R^2	HepG2 IC_{50}[a] (nM)	Enzyme IC_{50}[b] (nM)
10		6.5	9.5
11		1.5	2.6
12		3.1	8.4
13		8.8	NT

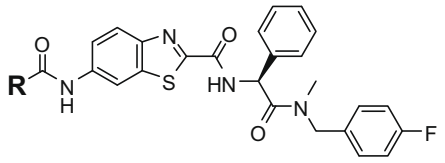
[a] In the HepG2 assay, the IC_{50} for the inhibition of ApoB secretion was determined in triplicate measurements, with individual variation of <15%.

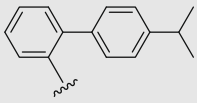
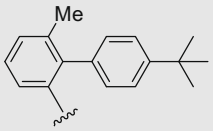
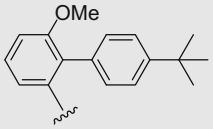
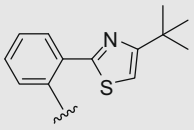
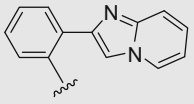
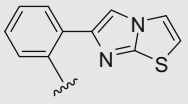
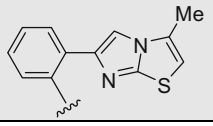
[b] In the enzyme assay, the commercially available kit from Chylos was used. The IC_{50} was determined in triplicate measurements, with individual variation of <15%. For comparison purposes, dirlotapide had an IC_{50} of 2 nM in the enzyme assay and 4 nM in the HepG2 assay.

ortho position of the lower phenyl ring, as in **15** and **16**, did not affect the MPT activity significantly. Compound **17**, with a 4-*tert*-butylthiazole group instead of a 4-*tert*-butylphenyl group,[13] was still a potent MTP inhibitor, with an IC_{50} of 4.0 nM in the HepG2 assay. Compounds **18–20** all contained a fused bicyclic ring in this portion of the molecule.[14] Compound **18**, with an imidazopyridine ring, had an IC_{50} of 4.2 nM in the HepG2 assay. Compound **19**, with an imidazothiazole ring, was less active in the HepG2 assay, with an IC_{50} of 23.7 nM. Incorporating a methyl group onto the imidazopyridine, as in **20**, regained some of the MTP activity, and an IC_{50} of 6.9 nM was obtained in the HepG2 assay.

All compounds shown in Tables 1 and 2 were evaluated for in vivo efficacy in the mice 3-day food intake study. In this type of study, lean mice were placed on a high fat diet for 1 week. Animals were then dosed with the MTP inhibitor in the evening, once daily, for 3 consecutive days. Food intake and body weight were taken daily. At the conclusion of the study, mice were dosed with the MTP inhibitor in the morning and blood was withdrawn at 30 min, 2 h, and 6 h and analyzed for triglycerides and PYY levels. Efficacious compounds are defined as those that could lower food intake, body weight and plasma triglycerides and at the same time increase PYY levels. Compounds **2**, **14–21** were evaluated in this 3-day food intake study. Of these nine compounds, compound **14** was the most efficacious one, with oral activity observed at doses as low as 3 mg/kg when administered orally.[15] At the 3 mg/kg dose, there was a 11% decrease in plasma triglycerides at the 6 h time point. At the higher dose of 10 mg/kg, there was a corresponding 45% decrease in plasma triglycerides level at the same time point. As shown in Figure 1, statistically significant increase in PYY levels was observed at both the 3 and 10 mg/kg doses. Consistent with the increase level of PYY, there was a marked decrease in average total food intake per group. At the 3 mg/kg dose, there was a 14% decrease in average total food intake per group, when com-

Table 2
MTP inhibitors with varying carboxamide groups at the C6 position of the benzothiazole



Compound	R	HepG2 IC_{50}[a] (nM)	Enzyme IC_{50}[b] (nM)
14		6.2	11.2
15		2.9	4.7
16		2.4	7.9
17		4.0	4.6
18		4.2	13.0
19		23.7	14.8
20		6.9	16.9

[a] In the HepG2 assay, the IC_{50} for the inhibition of ApoB secretion was determined in triplicate measurements, with individual variation of <15%.
[b] In the enzyme assay, the commercially available kit from Chylos was used. The IC_{50} was determined in triplicate measurements, with individual variation of <15%.

pared with the vehicle group. And at the 10 mg/kg dose, there was a 19% decrease in average total food intake per group. Along with the decrease in food intake, there was decrease in body weight. In this short 3-day food intake study, there was a 3% decrease in body weight at the 3 mg/kg dose and a 4% decrease in body weight at the 10 mg/kg when compared with the vehicle group.

In a longer 28-day study involving diet-induced obesity (DIO) mice, there was a much more significant loss in body weight between the two different doses. Prior to the study, C57BL/6 male mice were put on a high fat diet for 4 weeks to achieve the desired weight of about 40 g. The animals remained on a high fat diet for the duration of the study. Mice, (n = 10), were then dosed once a day for 4 weeks with compound **14** at either 1 or 3 mg/kg po. The amount of food given was weighed and replaced with preweighed food 3 times a week so that the food intake can be taken

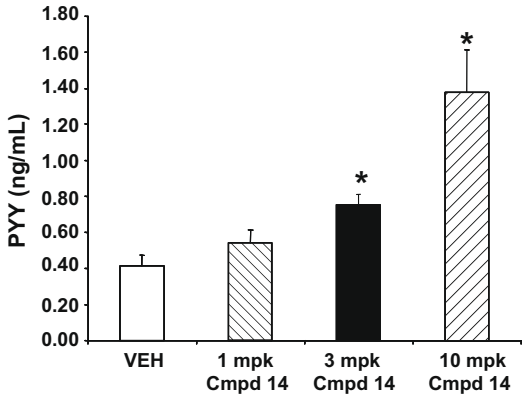


Figure 1. PYY level after a 3-day food intake study in mice fed with a high fat diet. Mice (36-C57BL/6 male, n = 9) were dosed either with the vehicle (5% PEG400, 0.25% Tween 80, and 94.25% H_2O) or with compound **14** (1, 3, or 10 mg/kg po). PYY was analyzed using the EIA Kit from Phoenix Pharma. *p < 0.02 (Student's t test).

and compared with the weight loss throughout the study. In this study there was a fed blood glucose test done at the start of the study to establish the baseline, and then at week 2 and week 4. At the end of the study, the necessary plasma samples were taken for a reading on the insulin levels.

DIO mice showed a fairly quick response to compound **14**; and there was steady decrease in food intake by the 4th day of the study. By day 21, there was a 12% decrease in total food intake per group for the 3 mg/kg dose and a corresponding 9% decrease for the 1 mg/kg dose. Along with the reduction in food intake was a nice reduction in body weight. Animals dosed with 3 mg/kg of compound **14** experienced a 3% decrease in body weight; and animals dosed with 1 mg/kg of compound **14** showed a 1% gain in body weight. This compared favorably with the vehicle group, which showed a gain of 10% in body weight (see Fig. 2).

Along with the decrease in body weight was a reduction in glucose levels. At the 2-week period for instance, there was a statistically significant decrease in fed glucose levels. Similar reduction in fed glucose was also observed at the end of the study. More importantly, the insulin levels at the conclusion study showed a statistically significant decrease at the 3 mg/kg dose (see Fig. 3). Plasma

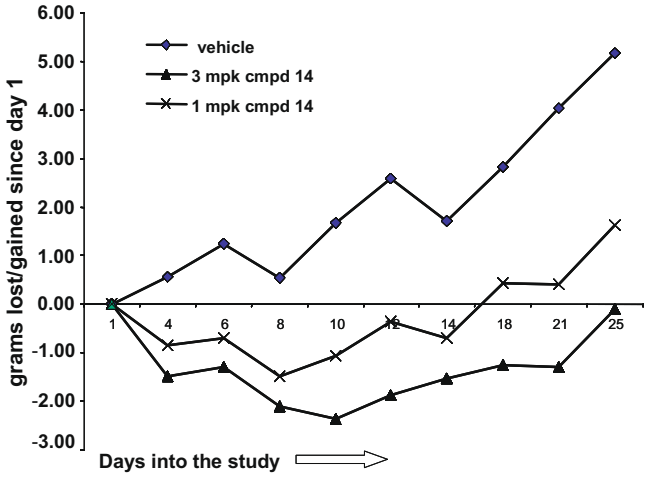


Figure 2. Changes in body weight in the 28-day mice diet-induced obesity (DIO) model. C57BL/6 male mice were placed on a high fat diet for 4 weeks prior to the study to achieve a body weight of about 40 g. Animals (n = 10) were dosed with either the vehicle (5% PEG400, 0.25% Tween 80, and 94.25% H_2O) or with compound **14** (1 or 3 mg/kg po).

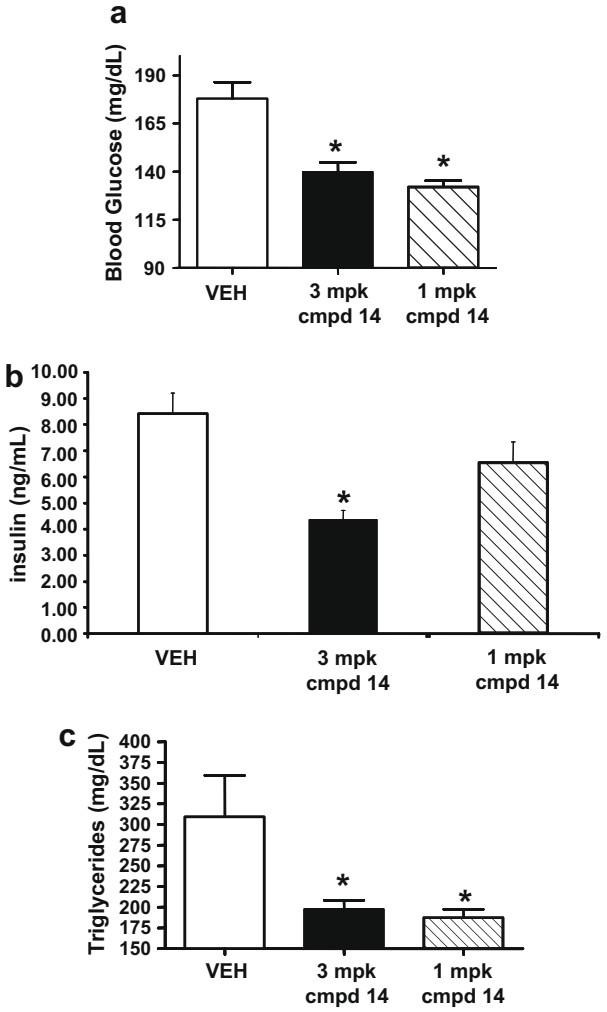


Figure 3. Plasma glucose, insulin and triglycerides levels from the 28-day mice DIO study. (a) Fed plasma glucose level after 2 weeks; (b) plasma insulin levels (4 weeks); (c) plasma triglycerides levels (4 weeks). Statistics were conducted as an ANOVA, with $^{*}p < 0.05$.

triglycerides levels and total cholesterol levels were also down significantly after 4 weeks. In these DIO animals, the drop in plasma triglycerides levels was much more significant than in the previous 3-day food intake study. Liver enzymes (ALT, AST, and ALK phosphatase) were normal for these DIO animals after they have been dosed with compound **14** for 4 weeks. There was also no increase in liver weight for animals that have been dosed with compound **14** for 4 weeks.

In terms of PK properties, compound **14** possesses the desirable low systemic exposure. In a rat PK study, with an IV bolus injection of compound **14** at 1 mg/kg, the systemic clearance was determined to be 1.03 L/h/kg (see Table 3).[16] The half-life ($T_{1/2}$) was 2.0 h; the volume of distribution was 2.99 L/kg; the C_{max} was 5982.69 μg/L, and the $AUC_{(0-\infty)}$ was 975.77 μg/L h. Following a 3-mg/kg po dose, the C_{max} and T_{max} were 22.16 μg/L and 0.50 h, respectively. The oral half-life ($T_{1/2}$) was 2.13 h and the $AUC_{(0-\infty)}$ was 40.76 h μg/L. Based upon these parameters, the oral bioavailability for compound **14** in rat was determined to be rather low (%F = 1.29%). A dose proportionality study was carried out to further assess the plasma and liver concentration of compound **14**. Here, rats (n = 3) were dosed at 3, 10, 30, and 100 mg/kg orally; liver and plasma samples were collected at the 0.5, 1, 2, 4, and 8 h time points and analyzed for drug concentration. At the 3 mg/kg dose, the plasma C_{max} was 35.01 μg/L and the $AUC_{(0-\infty)}$ was 109.3 μg/L h. There was essentially no dose proportionality and at the 10 and 30 mg/kg dose, the corresponding plasma C_{max} was 57.97 μg/L and 49.46 μg/L, respectively. Even at the highest dose of 100 mg/kg, the plasma C_{max} was just slightly higher at 69.50 μg/L and the $AUC_{(0-\infty)}$ was 597.77 μg/L h. In terms of liver concentration, at the 3 mg/kg dose, the liver C_{max} was 56.97 μg/kg and the $AUC_{(0-\infty)}$ was 1218.27 μg/kg h. Even at the highest dose of 100 mg/kg, the liver C_{max} was just slightly higher at 84.04 μg/kg and the $AUC_{(0-\infty)}$ was 1775.40 μg/kg h. These lower drug concentrations in the liver indicated that compound **14** had the desired low systemic exposure that we were looking for in an enterocyte-specific MTP inhibitor. This level of systemic exposure is lower than that reported by dirlotapide.[7]

Table 3
Selected PK parameters for compound **14** in rats

Plasma	$AUC_{(0-\infty)}$ (μg/L h)	C_{max} (μg/mL)	$T_{1/2}$ (h)
PO-3 mpk	109.30	35.01	2.42
PO-10 mpk	224.35	57.97	7.91
PO-30 mpk	510.44	49.46	10.45
PO-100 mpk	597.77	69.50	5.44
Liver	$AUC_{(0-\infty)}$ (μg/kg h)	C_{max} (μg/kg)	$T_{1/2}$ (h)
PO-3 mpk	1218.27	56.97	10.66
PO-10 mpk	2640.65	90.06	19.40
PO-30 mpk	3027.45	73.11	23.68
PO-100 mpk	1775.40	84.04	6.44

Sprague–Dawley rats (n = 3) were dosed with **14** at 3, 10, 30, and 100 mg/kg po; liver and plasma samples were collected at the 0.5, 1, 2, 4, and 8 h time points and analyzed for drug concentration.

In summary, a series of benzothiazole derivatives has been shown to be potent MTP inhibitors. Compound **14** was able to reduce food intake along with body weight and thus lowered the plasma triglycerides, glucose, and insulin levels in the 28-day mice DIO study at doses as low as 3 mg/kg po. Extensive rat PK studies further confirmed that compound **14** had a low systemic exposure. The ability to selectively inhibit MTP only in the enterocytes and not in the liver could help minimize the risk of liver toxicity that was commonly associated with previous systemic MTP inhibitors.

Acknowledgments

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15. Compounds **2**, **15**, and **16** showed oral activity only at 10 mg/kg. Compounds **17**–**21** were not orally active at 10 mg/kg.
16. The IV vehicle was 5% EtOH, 5% Cremophor, and 90% water.