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Discovery of potent and orally active MTP inhibitors as potential anti-obesity agents

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Abstract—We have successfully identified a number of novel MTP inhibitors with single digit nanomolar potency. Analogues **10aq** and **10dq** demonstrated in vivo efficacy in a murine gut retention assay.
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As in human health, obesity is a growing health problem in companion animals, with 25–40% of the pet population estimated to be overweight and 5–10% considered severely obese.¹ Obesity predisposes dogs and cats to a number of harmful conditions including diabetes, hepatic lipidosis, cancer, osteoarthritis, dermatitis and musculoskeletal problems such as cruciate and inter-vertebral disk rupture. Obesity also negatively impacts veterinary patients with cardiovascular and respiratory disease and limits the efficacy of pharmaceutical therapy in these conditions.^{2–11} Current therapy for obesity is based on food restriction and/or exercise and affords limited success in most patients. The failure of weight loss programs is largely the result of poor owner compliance due to hunger and begging of the pet. Because there are no veterinary drugs currently available for the treatment of obesity, there is a major opportunity for a safe, efficacious agent.

Microsomal triglyceride transfer protein (MTP)¹² is involved in the assembly of triglyceride-rich chylomicrons in enterocytes and very low-density lipoproteins (VLDL) in hepatocytes.^{12–14} MTP is located in intestinal and liver tissues where it plays a role in

lipid assembly and transport.¹² Inhibition of MTP has been shown to be an effective method for reducing serum cholesterol.¹⁵ Recently we disclosed the use of MTP inhibitors for the treatment of obesity by inhibition of fat absorption.¹⁶

Several potent MTP inhibitors have been disclosed, including CP-346086 (**1**),¹⁷ implitapide (**2**),^{18,19} JNJ-4506463 (**3**),²⁰ diaminoindane derivative²¹ (**4**) and BMS-212122 (**5**).²² Starting from **1** as a lead, we successfully identified a new class of potent MTP inhibitors, represented by the indole amide **6** (Fig. 1).²³ In order to further explore the chemical space and ADME properties in this series many analogs have been prepared by either replacing the indole moiety with other fragments or varying the terminal amines. In this paper, we would like to disclose the syntheses and SAR of phenyl/substituted phenyl moieties.²⁴ This research effort resulted in the discovery of a number of highly potent MTP inhibitors for the potential treatment of obesity, highlighted by analog **10dq** (entry 35, Table 1).

Two factors were considered in replacing the indole fragment in **6**: (1) the rigidity and (2) the size of the new fragments. A parallel synthesis approach was employed in order to quickly explore the SAR of the new templates. As depicted in Figure 2, the acid derivatives **8a–8i** were chosen to replace the indole moiety in **6** based on the considerations mentioned above.

Keywords: MTP; Microsomal triglyceride transfer protein; Obesity.

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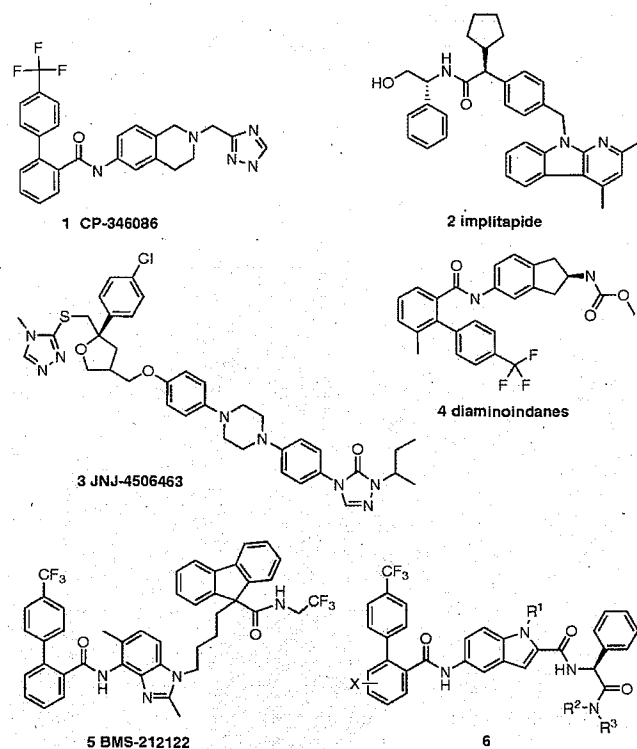


Figure 1. Selected MTP inhibitors.

A diverse set of amines was selected in order to quickly explore SAR (Fig. 3).

The preparation of the analogues is outlined in Scheme 1. The 4'-(trifluoromethyl)-2-biphenylcarboxylic acid (**7**) was reacted with **8a–8i** to provide the ester intermediates, which were then hydrolyzed under basic conditions to furnish the acids (**9a–9i**). The phenylglycine derivatives **13j–13r** were prepared by coupling Boc-protected phenylglycine **11** with amines **12j–12r**. Several standard amide coupling reaction conditions were screened in order to avoid epimerization of the chiral center of the phenylglycine. The coupling condition, PyBroP/DIPEA/DCM, proved to be the most robust for the coupling process without epimerization as monitored by chiral HPLC. Subsequently coupling the acid **9a–9i** with the phenylglycine derivatives **13j–13r** provided the final analogues represented by **10** for biology screening Table 2.

All analogues were tested in a canine MTP in vitro binding assay.²⁵ The results are summarized in Table 1. In general, analogues prepared from the mono-aryl templates (**8a–8d**, entries 1–36) demonstrated good in vitro potency despite their decreased size compared to the indole analog **6**. The analogues derived from 3-methoxy **8c** and 3-methyl **8d** templates showed the most potent inhibition toward MTP, suggesting a lipophilic binding pocket for these substituents. An electron-donating group on the 2-position of the phenyl ring was tolerated (entries 19–27). Electron

Table 1. In vitro canine MTP inhibition data

Entry	Compound	NH ₂ -x-COOH	NR ¹ R ²	MTP inhibition IC ₅₀ (nM)
1	10aj		12j	16.93
2	10ak		12k	2.53
3	10al		12l	22.46
4	10am		12m	29.51
5	10an		12n	69.39
6	10ao		12o	35.40
7	10ap		12p	12.97
8	10aq		12q	17.38
9	10ar		12r	13.29
10	10bj		12j	20.51
11	10bk		12k	ND
12	10bl		12l	13.27
13	10bm		12m	6.23
14	10bn		12n	14.78
15	10bo		12o	1.01
16	10bp		12p	23.13
17	10bq		12q	6.47
18	10br		12r	5.28
19	10cj		12j	
20	10ck		12k	2.0
21	10cl		12l	3.85
22	10cm		12m	6.38
23	10cn		12n	ND
24	10co		12o	1.68
25	10cp		12p	7.14
26	10cq		12q	1.75
27	10cr		12r	2.34
28	10dj		12j	ND
29	10dk		12k	1.64
30	10dl		12l	3.50
31	10dm		12m	ND
32	10dn		12n	ND
33	10do		12o	1.78
34	10dp		12p	4.62
35	10dq		12q	3.37
36	10dr		12r	
37	10ej		12j	85.5
38	10ek		12k	5.53
39	10el		12l	26.8
40	10em		12m	29
41	10en		12n	72.7
42	10eo		12o	ND
43	10ep		12p	26.6
44	10eq		12q	14.9
45	10er		12r	18.3
46	10fj		12j	15.6
47	10fk		12k	61.15
48	10fl		12l	2.97
49	10fm		12m	5.67
50	10fn		12n	11.78
51	10fo		12o	16.94
52	10fr		12r	4.11

Table 1 (continued)

Entry	Compound	NH ₂ -x-COOH	NR ¹ R ²	MTP inhibition IC ₅₀ (nM)
55	10gj		12j	17.27
56	10gk		12k	35.92
57	10gl		12l	>100
58	10gm		12m	55.84
59	10gn		12n	>100
60	10go		12o	69.84
61	10gp		12p	7.33
62	10gq		12q	8.27
63	10gr		12r	ND
64	10hj		12j	>100
65	10hk		12k	>100
66	10hl		12l	>100
67	10hm		12m	>100
68	10hn		12n	>100
69	10ho		12o	>100
70	10hp		12p	>100
71	10hq		12q	>100
72	10hr		12r	>100
73	10ij		12j	23.56
74	10ik		12k	96.71
75	10il		12l	69.52
76	10im		12m	95.89
77	10in		12n	>100
78	10io		12o	>100
79	10ip		12p	76.52
80	10iq		12q	>100
81	10ir		12r	9.43

ND, not determined.

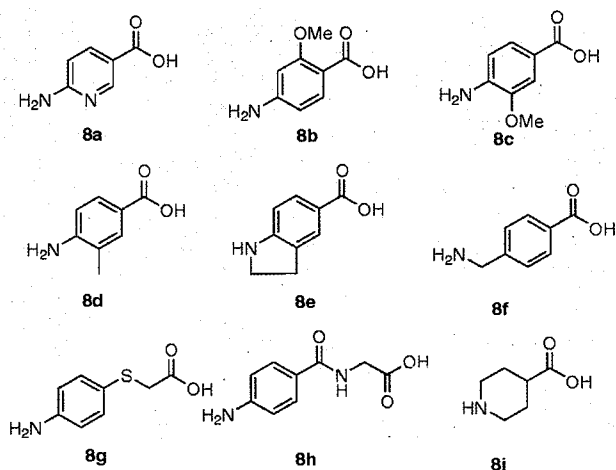
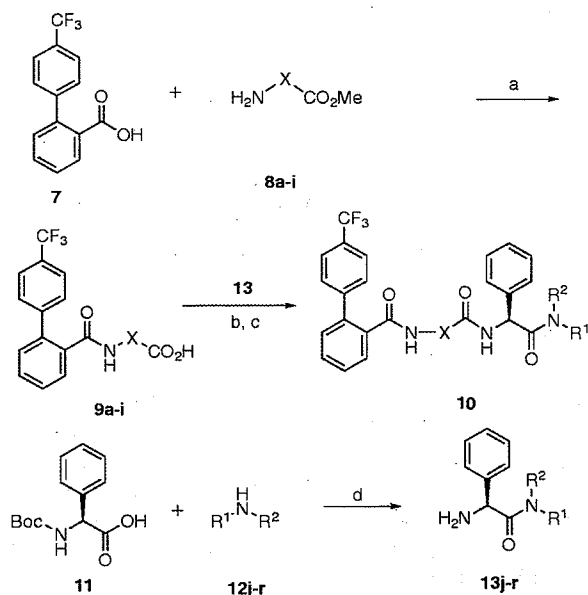
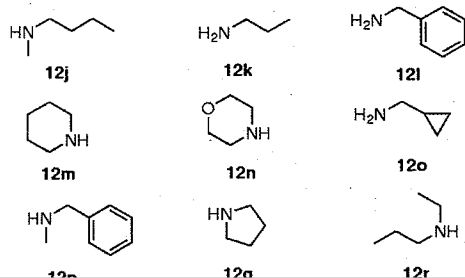


Figure 2. Acid derivatives.



Scheme 1. Reagents and conditions: (a) i—PyBroP, DIPEA, DCM, 0 °C to rt, ii—LiOH, THF/H₂, reflux, >95%; (b) EDC, HOBT, DIPEA, DCM, rt, >85%; (c) LiOH, THF/H₂O, reflux, >98%; (d) i—PyBroP, DIPEA, DCM, 0 °C to rt, ii—4 N HCl/dioxane, 100%.

Table 2. In vivo data for compounds 10aq and 10dq

Entry	Compound	ED ₂₅ (mg/kg, rat)
8	10aq	6.59
35	10dq	3

(entries 10–36). When a conformationally restricted template 8e was used, all analogues prepared showed a significant drop in potency toward MTP. Templates in which the aniline functionality was replaced with more flexible benzylic amines (8f, 8g, 8h and 8i) were in general less potent toward MTP.

Several potent analogs were progressed into in vivo studies. The murine gut retention assay²³ was used to assess a compound's ability to inhibit intestinal MTP. In this assay, compounds 10aq and 10dq were potent inhibitors of intestinal MTP, with ED₂₅s of 6.93 and 3 mg/kg, respectively.

In summary, we have successfully identified a number of novel and potent MTP inhibitors. Analogues 10aq and 10dq also demonstrated in vivo activity when tested in a murine gut retention assay.

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25. Canine in vitro MTP assays. (A) Canine hepatic microsomes isolation: canine microsomes are first isolated from canine liver by thawing frozen liver on ice and rinsing several times with 0.25 M sucrose. A 50% liver homogenate (w/v) is made in 0.25 M sucrose. The homogenate is diluted 1:1 with 0.25 M sucrose, and centrifuged at 10,000g for 20 min. The supernatant is saved. The pellet is re-suspended

in a minimal volume of 0.25 M sucrose and re-centrifuged at 10,000g for 20 min at 4 °C. The supernatants are combined and centrifuged at 105,000g for 75 min at 4 °C. The supernatant is discarded and the resulting microsomal pellet is saved. The microsomal pellet is re-suspended in a minimum volume of 0.25 M sucrose and diluted to 3 ml/g liver weight in 0.15 M Tris-HCl, pH 8.0. The resulting suspension is divided into 12 tubes and centrifuged at 105,000g for 75 min. The resulting microsomal pellets are stored at -80 °C until needed. MTP is isolated by thawing the microsomal pellet tube and suspending in 12 ml/tube of cold 50 mM Tris-HCl, 50 mM KCl, 2 mM MgCl, pH 7.4, and slowly adding 1.2 ml of a 0.54% deoxycholate, pH 7.4 solution. After 30 min incubation on ice with gentle mixing, the solution is centrifuged at 105,000g for 75 min at 4 °C. The supernatant, containing soluble MTP, is dialyzed for 2–3 days with 5 changes of assay buffer (15.0 mM Tris-HCl, 40 mM NaCl, 1 mM EDTA, 0.02% NaN₃, pH 7.4). (B) MTP activity assay reagents: donor liposomes are created by adding 447 mM egg phosphatidylcholine (68/20 ml), 83 mM bovine heart cardiolipin (20 ml) and 0.91 mM [¹⁴C]triolein (110 Ci/mol) (20/20 ml). The lipids are available in chloroform and are first dried under nitrogen and then hydrated in assay buffer to the volume needed. To create liposomes, lipids are sonicated for ~7 min. Lipids are centrifuged at 105,000g for 2 h and liposomes are harvested by removing the top ~80% of supernatant into separate tube. Acceptor liposomes are created by adding 1.33 mM egg phosphatidylcholine (404/40 ml), 2.6 mM triolein (100/40 ml) and 0.5 nM [³H]egg phosphatidylcholine (50 Ci/mol) (10/40 ml). The lipids are available in chloroform and are first dried under nitrogen and then hydrated in assay buffer to the volume needed. To create liposomes, lipids are sonicated for ~20 min. Lipids are centrifuged at 105,000g for 2 h and are harvested by removing the top ~80% of supernatant into separate tube. (C) MTP in vitro lipid transfer inhibition assay: appropriately diluted drug or control samples in donor assay buffer containing 5% BSA are added to reaction tubes containing assay buffer, 50 ml donor liposomes, 100 ml acceptor liposomes, and partially purified liver MTP. The tubes are vortexed and incubated on a tube shaker for 1 h at 37 °C to allow lipid transfer reaction to occur. Donor liposomes are precipitated by adding 300 ml of a 50% (w/v) DEAE cellulose suspension in assay buffer to each tube, followed by gentle/repeated inversion for 5 min at room temperature. Tubes are then centrifuged at ~1000 rpm to pellet resin. Four hundred milliliters of supernatant is transferred into a scintillation vial with scintillation fluid and DPM counts for both [³H] and [¹⁴C] are determined. Triolein transfer is calculated by comparing the amount of [¹⁴C] and [³H] remaining in the supernatant to [¹⁴C] and [³H] in the original donor and acceptor liposomes, respectively. % Triolein transfer = ($\frac{[^{14}\text{C}]_{\text{supernatant}}}{[^{14}\text{C}]_{\text{original donor}}}$) × ($\frac{[^3\text{H}]_{\text{acceptor}}}{[^3\text{H}]_{\text{supernatant}}}$) × 100. IC₅₀ values are obtained using standard methods and first order kinetic calculations.