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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.

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] diaminohydroindan derivative[21] (**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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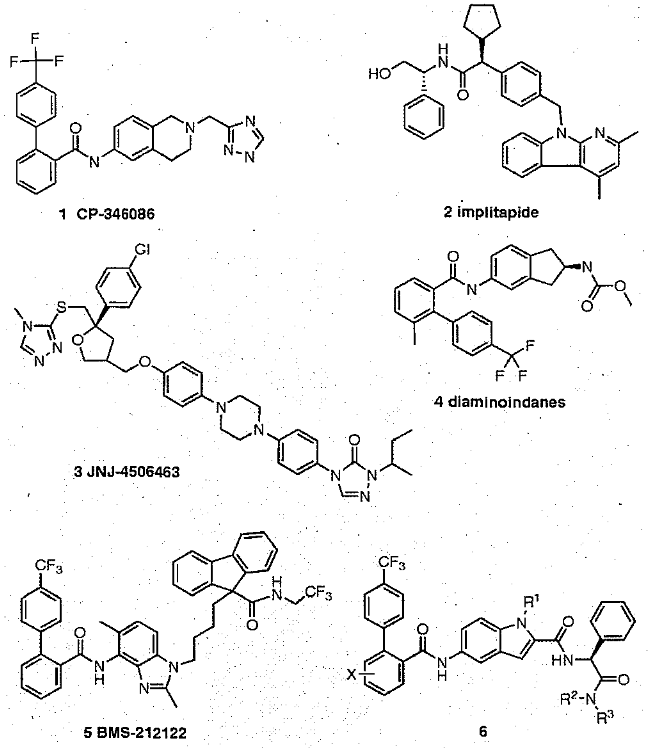


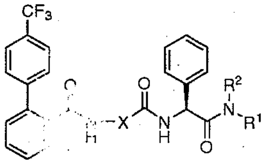
Figure 1. Selected MTP inhibitors.

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



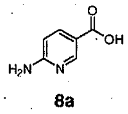
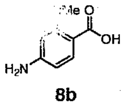
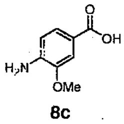
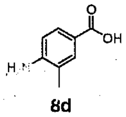
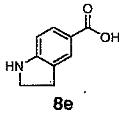
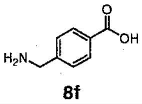
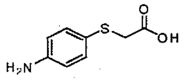
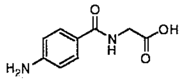
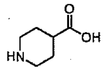
Entry	Compound	NH_2–x–COOH	NR^1R^2	MTP inhibition IC_{50} (nM)
1	**10aj**	 **8a**	**12j**	16.93
2	**10ak**		**12k**	2.53
3	**10al**		**12l**	22.46
4	**10am**		**12m**	29.51
5	**10an**		**12n**	69.39
6	**10ao**		**12o**	35.40
7	**10ap**		**12p**	12.97
8	**10aq**		**12q**	17.38
9	**10ar**		**12r**	13.29
10	**10bj**	 **8b**	**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**	 **8c**	**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**	 **8d**	**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**	 **8e**	**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**	 **8f**	**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

Table 1 (*continued*)

Entry	Compound	NH_2–x–COOH	NR^1R^2	MTP inhibition IC_{50} (nM)
55	**10gj**	 **8g**	**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**	 **8h**	**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**	 **8i**	**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.

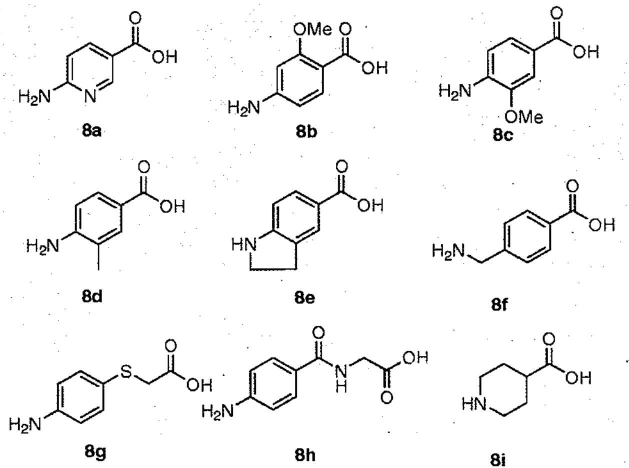
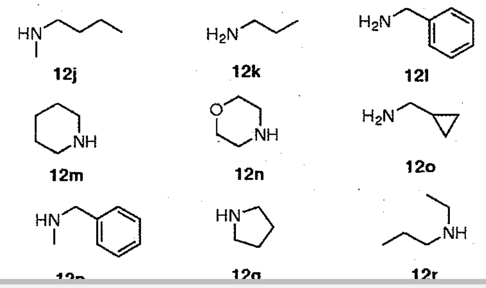
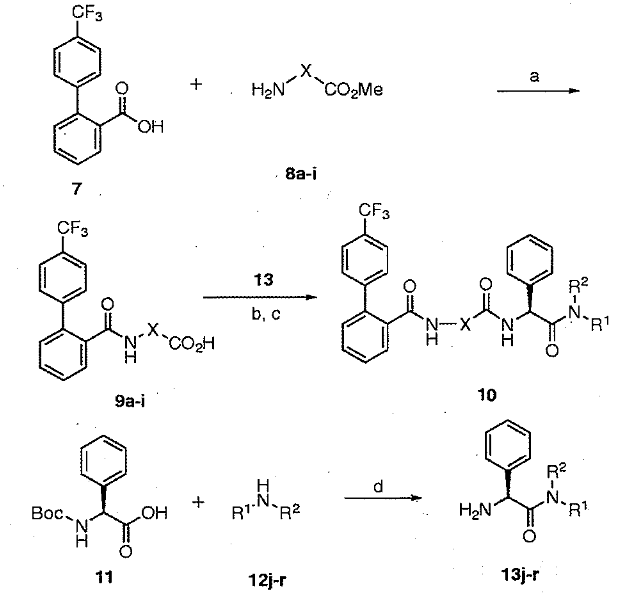


Figure 2. Acid derivatives.





Scheme 1. Reagents and conditions: (a) i—PyBroP, DIPEA, DCM, 0 °C to rt, ii—LiOH, THF/H_2, reflux, >95%; (b) EDC, HOBT, DIPEA, DCM, rt, >85%; (c) LiOH, THF/H_2O, 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 assay[23] 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 ED_{25}s 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 microsome 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 10,000*g* at 4 °C 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 10,000*g* for 20 min at 4 °C. The supernatants are combined and centrifuged at 105,000*g* 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 105,000*g* 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 105,000*g* 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% NaN$_3$, 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 ([illegible]/20 ml) and 0.91 mM [^{14}C]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 105,000*g* 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 [^{3}H]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 105,000*g* for 2 h and are harvested by removing the top ~80% of supernatant [illegible] tube. (C) MTP in vitro lipid transfer inhibition [illegible] appropriately diluted drug or control samples in [illegible] 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 for5 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 [^{3}H] and [^{14}C] are determined. Triolein transfer is calculated by comparing the amount of [^{14}C] and [^{3}H] remaining in the supernatant to [^{14}C] and [^{3}H] in the original donor and acceptor liposomes, respectively. % Triolein transfer = ([^{14}C]supernatant [illegible]) × ([^{3}H]acceptor/[^{3}H]supernatant) × 100 IC$_{50}$ values [illegible] obtained using standard methods and first order kinetic calculations.