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In vitro and in vivo profile of 5-[(4′-trifluoromethyl-biphenyl-2-carbonyl)-amino]-1*H*-indole-2-carboxylic acid benzylmethyl carbamoylamide (dirlotapide), a novel potent MTP inhibitor for obesity

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Abstract—The synthesis of a novel gut selective MTP inhibitor, 5-[(4′-trifluoromethyl-biphenyl-2-carbonyl)-amino]-1*H*-indole-2-carboxylic acid benzylmethyl carbamoylamide (dirlotapide), and its in vitro and in vivo profile are described. Dirlotapide (**3**) demonstrated excellent potency against MTP enzyme in HepG2 cells and canine hepatocytes. This novel MTP inhibitor also showed excellent efficacy when tested in a canine food intake model.

Obesity is a major health problem that has been associated with an increased incidence of type 2 diabetes mellitus, hypertension, and dyslipidemia.[1,2] Currently, obesity is the second leading cause of preventable death in the United States, resulting in more than 300,000 deaths per year.[3] In the US, more than 35% of the adult population is overweight (BMI 25–29), and 26% of the adult population is obese (BMI $\geqslant$30).[4] Over the past 10 years, the prevalence of obesity in the United States has increased by about 50% and a large component of this increase has been in children.[5,6] Weight loss is expected to have a clinically significant impact on the risk or selected co-morbidities (type 2 diabetes, cardiovascular disease, obesity associated quality of life issues, etc.). Currently there are only two branded anti-obesity agents on the market, orlistat (**1**, Roche, launched 1998)[7] and sibutramine (**2**, Abbott, 1997).[8,9] Microsomal triglyceride transfer protein (MTP)[10] is involved in the assembly of triglyceride-rich chylomicrons in enterocytes[10–12] and very low density lipoproteins (VLDL) in hepatocytes. MTP is located in intestinal and liver tissues where it plays a role in lipid assembly and transport.[10] A gut selective MTP inhibitor will inhibit transport of lipids within the endoplasmic reticulum without significantly decreasing serum triglyceride levels.[13] MTP inhibitors represent a unique class of anti-obesity agents that offer potent weight loss through appetite suppression[14] and intestinal fat mal-absorption.[15] In this paper, we would like to disclose the discovery of a potent and gut selective MTP inhibitor, dirlotapide (**3**), which is currently in clinical trials as an anti-obesity agent (Fig. 1).

The preparation of dirlotapide (**3**) is through a three-step linear synthesis and outlined in Scheme 1. Trifluoromethyl biphenyl carboxylic acid (**4**) was coupled with amino indole **5**[16] using EDC/HOBT coupling conditions to provide the ester **6**. Hydrolysis of the ester function under basic conditions furnished the acid **7** in high yield. The acid **7** was then coupled with phenylglycine derivative **8** to provide dirlotapide **3**.

As depicted in Table 1, dirlotapide (**3**) is a potent inhibitor of MTP as demonstrated by the inhibition of Apo B secretion from human HepG2 cells (IC_{50} = 1.5 nM) and in canine hepatocytes (IC_{50} = 2.9 nM).

The selectivity of dirlotapide (**3**) for MTP inhibition, relative to inhibition of other receptors in the body,

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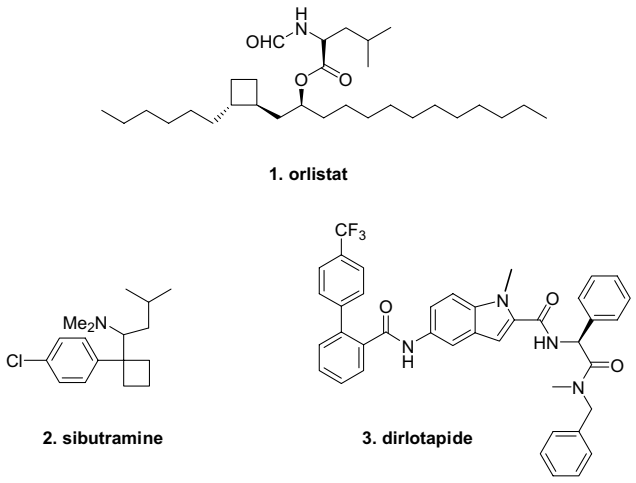
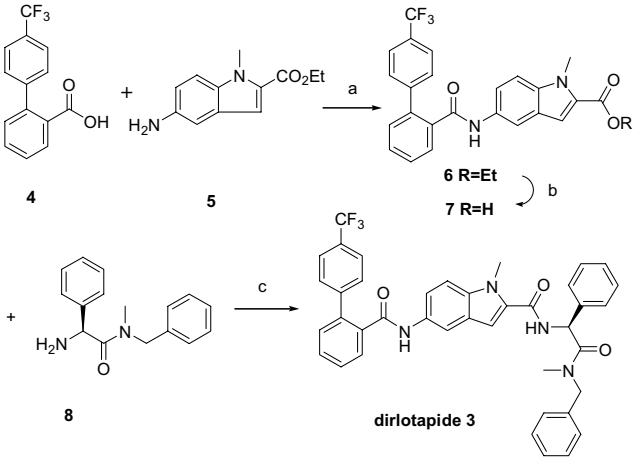


Figure 1. Selected anti-obesity drugs.

was demonstrated by a lack of activity (defined as $IC_{50} > 1$ μM) when tested at 1 μM against a broad range of selected peripheral and central receptors.[17] Since absorption by intestinal enterocytes is required for efficacy, but systemic exposure is not required, the ADME profile of dirlotapide **3** was assessed both in vitro and in vivo. Caco-2 cell permeability data of dirlotapide (**3**) in three-week-old cells are indicative of poor transcellular absorption ($P_{(app)A-B} < 1 \times 10^{-6}$ cm/s) with no indication of efflux (Table 2).

Dirlotapide (**3**) has moderate turnover in rat, dog, monkey, and human microsomes with projected hepatic extraction (ER) of 55%, 80%, 68%, and 35%, respectively. The microsomal and hepatocyte clearance are listed in Table 3.

The mean IC_{50} values of dirlotapide (**3**) for the inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A are all >30 μM. Microsomal incubations with specific CYP inhibitors (Table 4) were conducted to gain more quantitative information regarding the relative



Scheme 1. Reagents and conditions: (a) EDC, HOBT, DIPEA, DCM, rt, 85%; (b) LiOH, THF/H_2O, reflux, 4 h, 98%; (c) PyBroP, DIPEA, DCM, rt, 95%.

Table 1. In vitro MTP inhibition data[a]

Compound	Canine MTPi IC_{50} (nM)	Human $HepG_2$ MTPi IC_{50} (nM)
Dirlotapide (**3**)	2.9	1.5

[a] Values are averages of at least five determinations.

Table 2. Caco-2 data for dirlotapide (**3**)

Concn (μM)	+/− Ca^{2+}	pH	$P_{(app)A-B} \times 10^6$ cm/s	$P_{(app)B-A} \times 10^6$ cm/s
1	+	6.5/7.4	0.6 ± 0.0	0.4 ± 0.1
1	−	6.5/7.4	0.9 ± 0.3	—
10	+	6.5/7.4	0.4 ± 0.0	0.3 ± 0.1

Table 3. In vitro clearance for dirlotapide (**3**)

Species	Microsomal CL_{int} (mL/min/kg)	Microsomal CL_h (mL/min/kg)	Hepatocyte CL_h (mL/min/kg)
Rat	645	39	31.5
Dog	1091	28	—
Monkey	835	27	—
Human	91	7	7.8

Table 4. Inhibition of turnover in human liver microsomes using specific CYP inhibitors

Inhibitor	P450 inhibited	Concn (μM) inhibitor	Concn (μM) dirlotapide	$T_{1/2}$ (min)
—	—	—	0.5	48
Ketoconzole	3A4	1	0.5	369
Sulfaphenazole	2C9	5	0.5	47
(*S*)-Mephenytoin	2C19	200	0.5	70
Quinidine	2D6	5	0.5	36
Furafylline	1A2	5	0.5	45

contribution of each isoform to the overall CYP mediated metabolism of dirlotapide (**3**). These studies indicated that 3A is the predominant CYP mediated metabolic pathway for this compound.

Dirlotapide (**3**) binds significantly to plasma components with free fractions of 0.032%, 0.0088%, 0.026%, and 0.0097% in rat, dog, monkey, and human plasma, respectively (Table 5). Dirlotapide (**3**) has also been shown to have low red blood cell partitioning with blood-to-plasma concentration ratios of 0.64, 0.54,

Table 5. Nonspecific binding and red blood cell partitioning for dirlotapide (**3**)

Species	*N*	Concn (μM)	$f_u(b)$[b] (%)	BPR[a]
Rat	4	1.0	0.032 (0.022)[c]	0.64
Dog	4	0.2	0.0088 (0.0052)[c]	0.54
Monkey	6	0.2	0.026 (0.08)[c]	NA
Human	5	0.2	0.0097 (0.0020)[c]	0.61

[a] Blood/plasma concentration ratio.
[b] Free fraction (blood).
[c] Standard deviation.

Table 6. IV pharmacokinetic summary for dirlotapide (**3**)

Species	Rat			Dog			Monkey
Dose (mg/kg)	0.3	1	5	0.5	1	1[a]	0.5
Sex	M	M	M	M	M	M	M/F
N	3	4	2	3	3	4	3/3
CL_{plasma} (mL/min/kg)	92 (11)	37 (17)	14	5.5 (1.3)	4.7 (0.9)	7.6 (4.2)	12 (2.0)
V_{dss} (L/kg)	11 (4.6)	8.0 (3.9)	3.3	1.0 (0.21)	1.0 (0.20)	—	2.2 (0.5)
$T_{1/2}$ (h)	2.0 (0.6)	5.8 (4.1)	11.3	5	14 (1.0)	7.8 (3.4)	6.7 (0.6)
AUC(0–*t*) (ng h/mL)	51 (5.8)	485 (196)	5612	1494 (284)	3258 (678)	—	684 (118)
AUC(0–∞) (ng h/mL)	55 (5.9)	530 (235)	5892	1550 (316)	3614 (683)	2825 (1663)	703 (121)

[a] Study conducted in portal cannulated dogs.

Table 7. PO (fed) pharmacokinetic summary for dirlopatide (**3**)

Species	Rat			Dog		
Dose (mg/kg)	3	30	300	1[c]	0.5	5
Sex	M	M	M	M	M	M
N	3	4	2	4	3	3
Vehicle	a	a	b	d	e	e
$T_{1/2}$ (h)	6.3 (3.5)	6.3 (6.3)	NC	6.8 (1.9)	14.1 (5.0)	5.9 (1.8)
AUC(0–∞) (ng h/mL)	40.7 (8.4)	379 (152)	NC	289 (72.1)	663 (607)	1933 (197)
C_{max} (ng/mL)	4.7 (1.5)	21.0 (11.7)	27	26.8 (3.7)	31.1 (18.2)	250 (81)
T_{max} (h)	2.1 (0.0)	2.8 (1.5)	6	6.3 (2.0)	3.0 (1.0)	3.0 (1.0)
F (%)	2.6	2.3	1	10.6 (7.2)	43 (39)	12 (2)

(a) 80/20 PEG/saline; (b) PEG; (d) miglyol; and (e) miglyol/cremophor/water.
[c] Study conducted in portal cannulated dogs.

Table 8. Efficacy of dirlotapide (**3**) administered orally in obese Beagles for 3 months

		Baseline	Treatment	Change (%)
Food intake (g)	Placebo	282.2	282.3	−2
	Dirlotapide (**3**)	305.4	139.6	−54
Cholesterol (ng/mL)	Placebo	219	240	10
	Dirlotapide (**3**)	259	127	−51
Fecal fat (%)	Placebo	1.64	1.85	13
	Dirlotapide (**3**)	1.71	7.50	339

and 0.61 in rats, dogs, and humans, respectively (Table 5).

Intestinal selectivity should reduce the risk for adverse side effects. The in vivo selectivity of dirlotapide (**3**) for intestinal compared to liver MTP inhibition has been demonstrated in several animal models. In a murine model[16] investigating intestinal MTP inhibition, dirlotapide (**3**) demonstrated potent inhibition of intestinal fat absorption with an ED_{25} of 0.16 mg/kg. In contrast, it was a poor inhibitor of hepatic MTP as demonstrated in a murine model of triglyceride lowering, where it had an ED_{25} of 6 mg/kg.

The iv and oral pharmacokinetics of dirlotapide (**3**) were investigated in rats and dogs, and iv pharmacokinetics were investigated in monkeys. All pharmacokinetic parameters presented are derived from measurement of plasma concentrations. Tables 6 and 7 summarize the calculated pharmacokinetic parameters from the in vivo studies. Male rats had plasma clearance values ranging from 14 mL/min/kg at a dose of 5 mg/kg to 92 mL/min/kg at a dose of 0.3 mg/kg and moderate/high volume of distribution (3–11 L/kg). Following oral administration of 3 mg/kg the mean plasma C_{max} of 4.7 ng/mL was achieved at 2.1 h post-dose and the bioavailability (*F*) was 2.6%. At an oral dose of 30 mg/kg the resulting mean C_{max} was 21 ng/mL at 2.8 h post-dose and *F* was 2.3%. Between 3 and 30 mg/kg the systemic exposure increased in proportion to dose. However, increasing the dose to 300 mg/kg produced no further increase in systemic exposure when dosed as a solution in PEG 400. In male Beagle dogs the mean plasma clearance was 6.0 mL/min/kg and the mean volume of distribution was 1.0 L/kg (Table 6). Following oral administration to dogs dirlotapide (**3**) was moderately absorbed with oral bioavailability ranging from 12% to 43% (Table 7).

Robust anti-obesity efficacy was demonstrated in several canine studies with ad libitum feeding. In a 3-month efficacy study using medium chain triglyceride oil based solution, obese spayed and neutered beagles received individual dosages of dirlotapide (**3**) ranging from 0.15 to 0.5 mg/kg (average dose ~0.39 mg/kg). Doses were adjusted to limit the degree of weight loss to ~2%/week. The dogs lost a mean of 18.8% of body weight (1.5% per

week) compared to placebo animals that gained a mean of 10.6% body weight. In addition, average food intake was decreased by 54%, cholesterol decreased by 51%, and fecal fat increased from 1.86% to 7.50%. Following the weight loss phase, body weight was maintained in dogs receiving dirlotapide (**3**) over a 28-day period at dosages of 0.1–0.35 mg/kg (Table 8).

In conclusion, a very potent MTP inhibitor, dirlotapide (**3**), has been discovered and profiled. The robust efficacy has been shown in animal models. The unique ADME properties of dirlotapide (**3**) minimized the drug exposure systemically; therefore a better safety profile is expected in clinical trials.

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