Bioorganic & Medicinal Chemistry Letters 21 (2011) 4150–4154

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of microsomal triglyceride transfer protein (MTP) inhibitors with potential for decreased active metabolite load compared to dirlotapide

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ARTICLE INFO

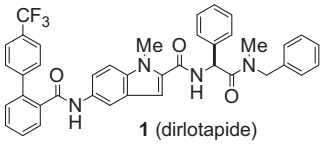
Article history:
Received 18 April 2011
Accepted 25 May 2011
Available online 2 June 2011

Keywords:
MTP
Obesity
Dirlotapide
Metabolite
Soft drug

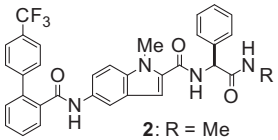
ABSTRACT

Analogues related to dirlotapide (**1**), a gut-selective inhibitor of microsomal triglyceride transfer protein (MTP) were prepared with the goal of further reducing the potential for unwanted liver MTP inhibition and associated side-effects. Compounds were designed to decrease active metabolite load: reducing MTP activity of likely human metabolites and increasing metabolite clearance to reduce exposure. Introduction of 4′-alkyl and 4′-alkoxy substituents afforded compounds exhibiting improved therapeutic index in rats with respect to liver triglyceride accumulation and enzyme elevation. Likely human metabolites of select compounds were prepared and characterized for their potential to inhibit MTP in vivo. Based on preclinical efficacy and safety data and its potential for producing short-lived, weakly active metabolites, compound **13** (PF-02575799) advanced into phase 1 clinical studies.

Dirlotapide (**1**) is a potent gut-selective inhibitor of microsomal triglyceride transfer protein (MTP).[1–4] As such it inhibits fat absorption by blocking the assembly of triglyceride-rich chylomicrons within enterocytes. Dirlotapide has demonstrated robust weight loss efficacy in animals. For example, in a three month efficacy study in dogs, a mean weight loss of nearly 20% was achieved.[1] The profound weight loss efficacy of dirlotapide is attributed not only to reduced fat absorption, but also to an anorectic effect, which is believed to result from the observed release of anorectic peptides (e.g., PYY and GLP-1) from fat-filled cells in the intestine. The compound is approved by the Food and Drug Administration for the treatment of obesity in dogs, marketed under the brand Slentrol.



1 (dirlotapide)



2: R = Me
3: R = H

Although dirlotapide also demonstrated significant weight loss efficacy in human clinical trials, serum transaminase elevations limited the dose and the efficacy that could be safely achieved.[5] Similar enzyme elevations have been reported for structurally diverse MTP inhibitors targeted to the liver for the treatment of atherosclerosis and hyperlipidemia. This effect is reversible and is believed to be mechanism-related, a consequence of triglyceride (TG) accumulation within the liver due to the inhibition of liver MTP.[6] Evidently, dirlotapide is not gut-selective in humans, even though only a small fraction of an orally administered dose is absorbed.

In humans, dirlotapide gives rise to metabolites, specifically the N-dealkylation products **2** and **3**, which accumulate and circulate at concentrations 2–7 fold higher than parent (unpublished results). These compounds also circulate as major metabolites in rats and dogs.[7] Since **2** and **3** retain activity against MTP (unbound potencies ~30-fold less than parent, Table 1), it was postulated that these contribute to the overall 'MTP inhibitory load' on the liver and to the associated serum liver enzyme elevations. That circulating active metabolites may contribute to the undesired side-effects of dirlotapide suggested strategies that, alone or in combination, could be applied in the design of new MTP inhibitors with improved gut selectivity: 1) designing compounds expected to give rise to metabolites with reduced activity against MTP, and 2) designing compounds expected to produce metabolites with increased clearance that do not accumulate. This letter describes the successful effort to identify compounds having the potential for increased gut selectivity based on these strategies.

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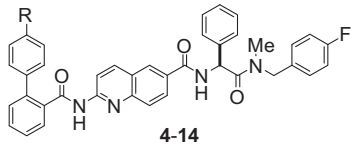
doi:10.1016/j.bmcl.2011.05.099

Table 1



4-14

	R	ApoB IC_{50}, apparent nM ± SD (n)	ApoB fraction unbound	ApoB IC_{50}, unbound pM	Dose mg/kg	Δ Food Intake (%) versus veh.[a]	Δ Body Wt% versus veh. [a]	Δ Liver TG (%) versus veh. [a]
1	N/A	2.7 ± 5.2 (154)	0.0042	11.3	10	−11 to −50	−8 to −117	70–216
2	N/A	1.8 ± 0.56 (3)	0.21	378	10	−40 (−43)	−106 (−115)	147 (112)
3	N/A	1.5 (1)	0.23	345	10	−37 (−43)	−108 (−115)	87 (112)
4	CF_3	0.74 (2)	0.0014	1.0	10	−43* (−11)	−96* (−8)	88* (70*)
5	H	2.8 (1)						
6	Me	2.2 (1)	0.0043	9.5	10	−38 (−50*)	−75* (−117*)	43 (216*)
7	Et	0.55 (2)						
8	*n*-Pr	0.44 (1)						
9	*i*-Pr	0.60 (2)	0.0011	0.7	10	−38* (−11)	−91* (−8)	16 (70*)
10	*t*-Bu	1.2 ± 3.8 (86)	0.00063	0.4	10	−60* (−46)	−123* (−97*)	unch. (120*)
					1[b]	−12 (−5)	−32 (−25)	unch. (38)
					3 (MED) [b]	−36* (−6)	−67* (−10)	unch. (22)
					10[b]	−33* (−8)	−96* (−32)	36 (102)
					30[b]	−41* (−37*)	−89* (−75*)	102 (136*)
					100[b]	−47* (−35*)	−107* (75*)	119* (152*)
11	OMe	3.0 (1)			10	−27 (−50)	−55 (−117)	
12	OEt	2.8 (1)						
13	*O*-*i*-Pr	0.77 ± 0.29 (15)	0.0038	2.0	10	−37* (−38*)	−56 (−95*)	16 (112*)
					3 (MED)	−23*	−65	unch.
					10	−30* (−28*)	−82 (−88*)	unch. (111*)
					30	−45*	−152*	15
14	*O*-*t*-Bu	0.83 (2)			10	−40* (−38*)	−108* (−95*)	72* (112*)

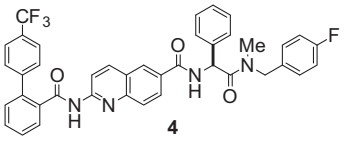
* Significantly different from vehicle ($P \leqslant 0.05$).

[a] In parentheses:% for compound **1**, run as positive control.

[b] 25% SDD formulation; compound **1** (comparator) 95% SDD.

For a primary screen, we used the well-established cell-based assay measuring the ability of compounds to inhibit apoB secretion from human hepG2 cells.[8] For select compounds, the extent of protein binding under the conditions of the apoB assay was measured by equilibrium dialysis[9], allowing free MTP potencies to be compared. To evaluate efficacy and the potential for gut selectivity, potent in vitro compounds were tested in rats to measure anorectic activity and liver TG accumulation. Thus, rats placed on a high fat (45%) diet were given daily oral doses of compound, administered in a self-emulsifying drug delivery system (SEDDS) vehicle for three days.[10] During this period, food intake was measured and afterwards, liver TG accumulation was determined and used to rank order compounds for the potential to produce liver enzyme elevations. In this model, even sub-efficacious doses of **1** produced statistically significant increases in liver TG.

Although there is no evidence to suggest structure-specific toxicity associated with dirlotapide, we chose as our chemical starting point the aminoquinoline analogue **4** that had been prepared as part of the original research effort leading to **1**. This compound displays very high potency against MTP and had the advantage of not falling within the scope of corporate 'structural alerts' developed as a guide to avoid possible class-related toxicity attributed to certain functional groups (in the case of dirlotapide, an aniline-like core). Like **1**, compound **4** showed no apparent therapeutic index between efficacy and elevation of liver TG in the rat model.

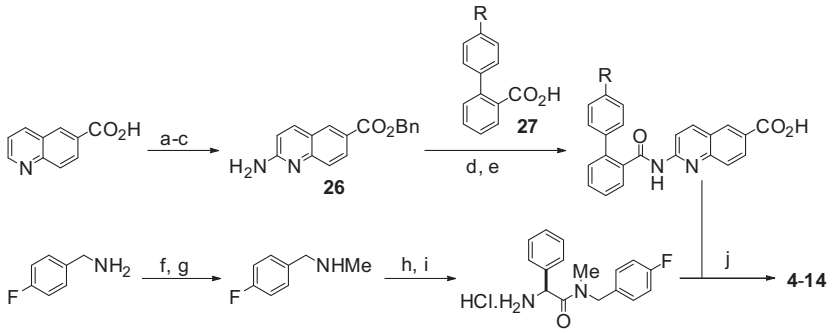


4

The dirlotapide metabolites **2** and **3** presumably accumulate due to a lack of 'soft sites' for further oxidative metabolism. Thus, our primary chemistry strategy was to incorporate an additional site of metabolism on the left hand portion of **4**. Even if N-dealkylation should occur first, the resulting metabolites would still be susceptible to further transformation to more polar, less active and more readily eliminated secondary metabolites. Modification of the 4′-trifluoromethyl group appeared particularly attractive with the reasonable expectation that lipophilic replacements for this group (e.g., alkyl, alkoxy) would retain MTP potency while polar groups (such as those produced by oxidative metabolism of 4′-alkyl and 4′-alkoxy) would show reduced activity against the target.

First we prepared a series of analogues of **4** (Scheme 1)[11] in which the 4′-trifluoromethyl group is replaced by hydrogen (**5**) or small alkyl groups (e.g., **6** to **10,** Table 1). Simple replacement of the 4′-trifluoromethyl group with hydrogen or alkyl provided compounds that retained potent MTP activity, and in the case of **6**, **9** and **10**, robust in vivo efficacy on par with or greater than **1** and **4**. Free potencies appeared to increase with alkyl chain size as suggested by the in vitro data for **6**, **9** and **10**. These three compounds showed potential for gut selectivity judging from reduced TG elevation relative to **1** and **4** at 10 mg/kg.

Next, we prepared compounds incorporating 4′-alkoxy groups (Scheme 1). With the exception of *O*-*t*-Bu (**14**), these offered the possibility that oxidative O-dealkylation would rapidly produce the corresponding phenol, which in turn would be susceptible to clearance via phase II metabolism. Based on the decreased in vivo efficacy of **11** relative to **1** (e.g., 27% reduction in food take versus 50% decrease for **1** at 10 mg/kg), 4′-OMe did not appear to be optimal. On the other hand, very promising compounds were identified from the 4′-*O*-*i*-Pr series, for example, **13**, which showed



Scheme 1. Reagents and conditions: (a) carbonyl diimidazole/EtOAc, then BnOH, 23 °C; (b) urea·H_2O_2/CH_2Cl_2/phthalic anhydride, 23 °C; (c) *p*-TsCl/CH_2Cl_2, then NH_4Cl/Et_3N, 23 °C; (d) **26**/EDC/DMAP/CH_2Cl_2, 23 °C; (e) LiOH·H_2O/MeOH/THF/H_2O, 23 °C; (f) EtOCHO/reflux; (g) $LiAlH_4$/THF, 0–23 °C; (h) *N*-BOC-L-phenylglycine/PyBrOP/*i*-Pr_2NEt/CH_2Cl_2, 23 °C; (i) HCl/dioxane, 0–23 °C; (j) EDC/Et_3N/DMAP/CH_2Cl_2, 23 °C.

Table 2

Dose mg/kg (po)	Δ Food Intake (%) versus veh.*	Δ Body Wt% versus veh.*	Δ Liver TG (%) versus veh.*	Δ ALT % versus veh.*	AUC ng h/mL
*Dirlotapide (**1**)*					
3	−11	−120	479*	122	37
10 (MED)	−36*	−269*	685*	1962*	75
30	−38*	−262*	770*	997*	331
*Compound **10***					
3 (MED)	−25*	−85	43	168	12
10	−51*	−255*	219*	143	42
30	−48*	−220*	212*	237	65
*Compound **13***					
10 (MED)	−21*	−82	113*	10	51
30	−25*	−148*	184*	191	47
100	−43*	−178*	303*	629*	170

* Significantly different from vehicle ($P \leqslant 0.05$).

efficacy equivalent to **1** and **4** and did not elicit appreciable liver TG elevations at 10 mg/kg.

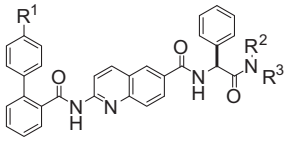
Based on their promising in vitro and in vivo profiles, compounds **10** and **13** were advanced to further define their efficacy and potential for improved gut-selectivity relative to **1**. Because the SEDDS formulation used for in vivo screening is not an acceptable formulation for human clinical trials, the compounds were formulated as 25% spray-dried dispersions (SDD) prior to carrying out definitive preclinical efficacy and safety studies.[12] Preliminary assessment of SDD-formulated **10** in the routine three-day rat model (Table 1) revealed good efficacy (minimally effective dose, MED = 3 mg/kg) and evidence of gut selectivity as previously observed using SEDDS. Next, SDD-formulated **10** and **13** were tested alongside **1** (95% SDD) in a seven-day rat model in which changes in food intake, body weight, liver TG, as well as serum alanine transaminase (ALT) were measured. In this definitive study, dose-effect relationships were examined (**1** and **10** dosed at 3, 10, and 30 mg/kg q.d., **13** dosed at 1, 3, 10, 30 and 100 mg/kg q.d.) and systemic exposures were measured (Table 2).

In agreement with the results in Table 1, compound **1** showed no evidence of gut selectivity in the definitive study, either on the basis of liver TG or serum ALT elevations. With respect to efficacy and ALT, the therapeutic index (TI) of **1** was ~1 based on dose and exposure. Although its minimally efficacious dose (MED) in the seven day study remained unchanged (3 mg/kg), compound **10** elicited statistically significant effects on liver TG at a lower dose than before (10 mg/kg) while compound **13** produced appreciable TG effects at its MED (10 mg/kg). Importantly, however, **10** showed no increase in ALT at any dose, while statistically significant increase of ALT was observed only at 100 mg/kg with **13**. On a dose basis, the TI's of compounds **10** and **13** with respect to efficacy and ALT are ~10-fold (30/3 and 100/10). Using systemic exposure data (AUC's), the TI's decrease to >5-fold (65/12) for **10** and ~3 fold (170/51) for **13**.

Metabolite identification studies for **10** and **13** in vitro (rat, dog, and human microsomes) and for **10** in vivo (rat and dog) confirmed that, as expected, both compounds undergo extensive CYP-mediated oxidation (hydroxylation or O-dealkylation) at the 4′-position accompanied by N-dealkylation (demethylation and debenzylation) of the terminal L-phenylglycinamide.[13] Importantly, for both compounds, the metabolite profile observed in rats was quite similar to that observed in human liver microsomes. Thus, provided the metabolites were shown not accumulate in vivo or were, in general, less active than parent drug, it would be reasonable to hypothesize that the improved TI observed in rats was due to reduced metabolite load compared to **1** and that the improved TI should translate to humans.

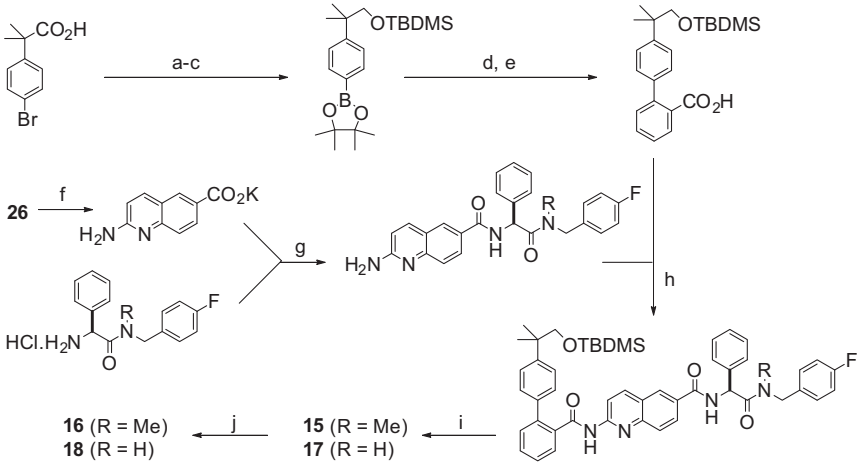
We prepared a number of metabolites of **10** and **13** as standards for PK studies and for in vitro characterization (Table 3, Schemes 2 and 3): **15**, **16**, **21**, **22**, **23**–formed from parent by 4′-oxidation; **19**, **20**, **24**, **25**–formed from parent by N-dealkylation, and **17**, **18**–formed from **10** by a combined 4′-oxidation and N-dealkylation.[14] With the exception of the N-demethylated metabolites **20** and **25** (which themselves are further metabolized at the 4′-position), all those profiled exhibited at least ~30-fold loss of free potency with respect to parent. As expected, the two metabolites formed by reaction at two sites (**17** and **18**) were less potent than the corresponding single-site product (compare **17** to **15** and **18** to **16**). Generally speaking, however, except for the carboxylic acids **18** and **23**, the loss of potency associated with metabolism at the

Table 3



Cmpd	R^1	R^2	R^3	ApoB IC_{50}, apparent nM ± SD (*n*)	ApoB fraction unbound	ApoB IC_{50}, unbound pM	Fold decrease free potency relative to parent
10 *and metabolites*							
10	*t*-Bu	Me	4-F-Bn	1.2 ± 3.8 (86)	0.00063	0.76	1
15	CMe_2CH_2OH[a]	Me	4-F-Bn	1.1 ± 0.35 (10)	0.023	25	33
16	CMe_2CO_2H[a]	Me	4-F-Bn	7.5 ± 2.2 (9)	0.0088	66	87
17	CMe_2CH_2OH	H	4-F-Bn	4.3 ± 0.52 (3)	0.0135 [b]	58	76
18	CMe_2CO_2H	H	4-F-Bn	12 ± 2.4 (3)	0.0211 [b]	253	333
19	*t*-Bu[a]	Me	H	0.56 ± 0.15 (12)	0.038	21	28
20	*t*-Bu	H	4-F-Bn	1.2 (1)	0.0023	2.8	4
13 *and metabolites*							
13	*O*-i-Pr	Me	4-F-Bn	0.77 ± 0.29 (15)	0.0038	2.9	1
21	OH	Me	4-F-Bn	11 (1)	0.0095	105	36
22	$OCH(Me)CH_2OH$	Me	4-F-Bn	9.4 (1)	0.013	122	42
23	$OCH(Me)CO_2H$	Me	4-F-Bn	22 (1)	0.022	484	167
24	*O*-i-Pr	Me	H	0.63 ± 0.14 (4)	0.160	101	35
25	*O*-i-Pr	H	4-F-Bn	1.21 ± 0.92 (3)	0.0061[b]	7.4	3

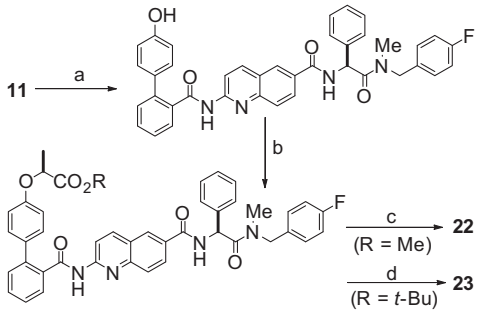
[a] Major circulating metabolite (>3% parent response) in rat and dog in vivo.
[b] Predicted using a computational model developed specifically for HepG2 apoB protein binding.



Scheme 2. Synthesis of 4′-metabolites of **10**. Reagents and conditions: (a) $LiAlH_4/Et_2O$, −78 °C, warm to 23 °C; (b) TBDMSCl/imidazole/CH_2Cl_2, 23 °C; (c) *n*-BuLi/THF, −78 °C, then 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, warm to 23 °C; (d) methyl 2-iodobenzoate/$Pd(PPh_3)_4/Cs_2CO_3$/DME, 100 °C; (e) $LiOH{\cdot}H_2O$/MeOH/THF/H_2O, 23 °C; (f) KOH/*i*-PrOH/H_2O, 85 °C; (g) EDC/THF, 23 °C; (h) EDC/DMAP/THF, 23 °C; (i) TBAF/AcOH/THF, 23 °C; (j) Jones reagent/acetone, 23 °C.

4′-position was not as dramatic as was hoped for, being in order of magnitude seen for metabolism of **1** to give **2** and **3**. Therefore, it was important to demonstrate a reduced capacity of metabolites of **10** and **13** to accumulate in vivo relative to dirlotapide metabolites **2** and **3**.

Indeed, following single dose oral administration of **10** (25% SDD) to rats (30 mg/kg) and beagle dogs (2.5 mg/kg), circulating exposures (AUC) of the major metabolites **15**, **16**, and **19** were 20% or less than that of parent in both species as determined using synthetic standards. The half-lives of these three metabolites in rats and dogs were approximately equivalent to parent due to formation rate limited clearance. Systemic exposure to parent drug was very low for **10** and **13** in both species (bioavailabilites <3%). We did not perform a parallel study with **13** to measure metabolite levels in vivo, making the reasonable assumption based on the sim-



Scheme 3. Synthesis of 4′-metabolites of **13**. Reagents and conditions: (a) BBr_3/CH_2Cl_2, −78 °C warm to 23 °C; (b) NaH/methyl or *t*-butyl 2-bromopropionate/DMF, 23 °C (c) $NaBH_4$/MeOH/THF, 55 °C; (d) TFA/CH_2Cl_2, 23 °C.

ilarity of structures that metabolites of **13** would behave similarly to those of **10** in vivo.

Based on their potential for an improved therapeutic index over dirlotapide (**1**) as demonstrated in preclinical models and the predicted reduced systemic exposure to MTP-active species (parent drug and metabolites), compounds **10** and **13** progressed to regulatory toxicology studies to support further development. Showing a clear safety advantage over **10** in regulatory safety studies, compound **13** (PF-02575799) advanced into human phase 1 clinical studies for treatment of obesity.

Acknowledgements

Thanks to David Griffith for review of the manuscript and very helpful comments.

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7. Compound **3** does not circulate as a major metabolite in dog. Dog has metabolic processes to limit circulating active metabolites.
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10. Male Sprague–Dawley rats (~300 g; $n = 5$ per group), acclimatized over seven days to a high fat diet (45% kcal from fat, Research Diets D01050602 M), received three consecutive daily doses of vehicle (SEDDS:H_2O 10:90) or compound in the same vehicle by standard oral gavage at 1.5 h prior to the dark cycle. The SEDDS vehicle was made up of Miglyol® 812 (10% by volume), triacetin (30%), polysorbate 80 (20%), and Capmul® MCM (30%).
11. Ref. 8 provides detailed experimental procedures for the preparation of **10** as well as spectroscopic data for compounds **4**, **6**–**14**, **19**, **20** and **25**.
12. Spray dried dispersions were prepared by spray drying a solution containing test compound and polymer (hydroxypropylmethylcellulose acetate succinate). For details, see Ref. 8
13. Metabolite identification was not performed for **13** in vivo. For both **10** and **13**, hydroxylation of the biphenyl group also occurred to afford phenolic metabolites, with and without accompanying 4′-oxidation and N-dealkylation. These were not profiled.
14. N-dealkylated metabolites **19**, **20**, **24** and **25** were prepared in a manner similar to that in Scheme 1 starting with the amide coupling of *N*-BOC-L-phenylglycine to methylamine hydrochloride (to afford **19** and **24**) or 4-fluorobenzylamine (to afford **20** and **25**).