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PRODRUGS OF CL316243: A SELECTIVE β_3-ADRENERGIC RECEPTOR AGONIST FOR TREATING OBESITY AND DIABETES

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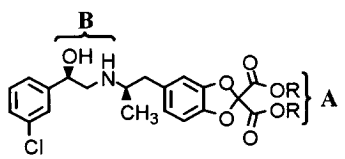
Abstract: CL316243 is a highly selective and potent β_3-adrenergic receptor agonist, and has been shown in rodent models to be an effective agent for treating obesity and Type II diabetes. To improve the oral absorption and pharmacokinetic profiles of CL316243, a number of prodrugs have been synthesized and evaluated. Several ester-type prodrugs show significant improvements in oral bioavailability in both rodent and primate models. © 1999 Elsevier Science Ltd. All rights reserved.

β-Adrenergic receptors (β-AR) belong to the superfamily of G-protein coupled receptors. Three subtypes of β-AR (β_1, β_2, and β_3) have been identified, and β_3-AR is implicated for the regulation of lipid metabolism.[1-3] The ability of β_3-AR agonists to stimulate adipocyte lipolysis and thermogenesis in brown adipose tissue (BAT) renders them potential antiobesity agents.[4] Since BAT also plays an important role in glucose homeostasis,[3] β_3-AR agonists may be useful for treating diabetes[5] as well. Recent discoveries of potent and selective human β_3-AR agonists have generated renewed interest in the development of these compounds for the treatment of obesity and diabetes.

CL316243, discovered in our laboratories,[6] is a highly selective and potent β_3-AR agonist in Phase II clinical trials. It has been shown, in rodent models, to be an effective agent for treating obesity and Type II diabetes. However, its clinical development is hampered by low oral bioavailability. In order to improve the oral absorption and pharmacokinetics profile of **CL316243**, we embarked on a prodrug program to synthesize and evaluate a large number of prodrug derivatives. Results of our investigation are reported herein.



CL316243 R = Na

Possible Handles for Prodrugs: **A and B**

We surmise that the high hydrophilicity of **CL316243** is a possible cause of low oral bioavailability. Therefore, our strategy to improve its oral absorption focuses on the development of prodrugs that increase the lipophilicity of the parent molecule. Although both the dicarboxylic acid (**A**) and aminoalcohol (**B**) functions could be used as handles for preparing prodrug derivatives, we concentrated most of our efforts in developing diester prodrugs of **CL316243**.

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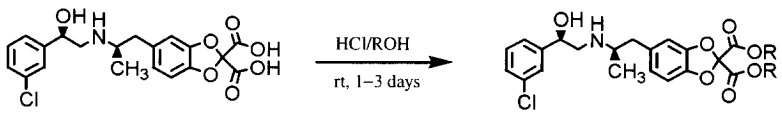
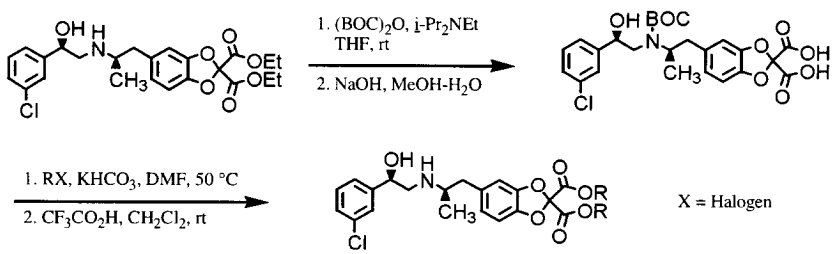
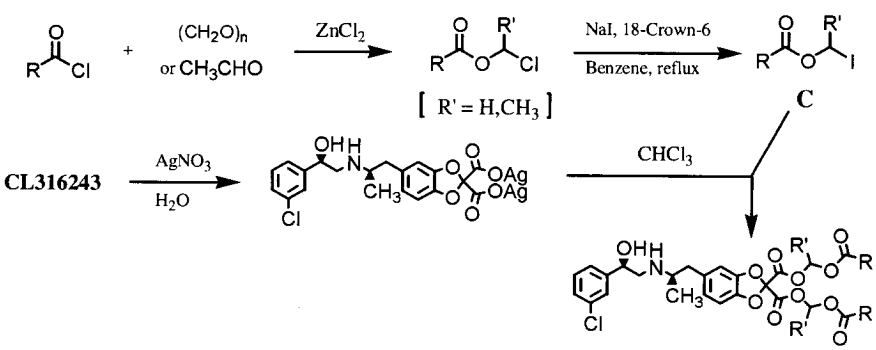
Five general classes of esters were investigated: (1) alkyl, (2) arylalkyl, (3) alkoxyalkyl, (4) acylmethyl, and (5) (acyloxy)alkyl. The alkyl, arylalkyl, and alkoxyalkyl diesters were prepared using either Scheme 1 or Scheme 2, depending on the availability of the corresponding alcohols, while acylmethyl diesters were synthesized according to Scheme 2. The synthesis of (acyloxy)alkyl diesters[7] is outlined in Scheme 3. In general, the di-silver salt of **CL316243** was prepared and alkylated with α-iodoalkyl esters (**C**)[8] at room temperature to give the double esters in good yields. All diesters were isolated as hydrochloride salts and used as such in the biological studies.



Scheme 1. Synthesis of Diesters by Fischer-Speier Esterification

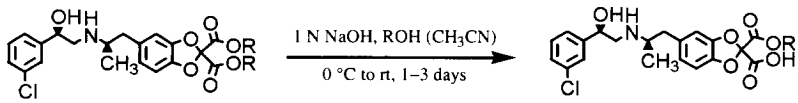


Scheme 2. Synthesis of Acylmethyl Diesters



Scheme 3. Synthesis of (Acyloxy)alkyl Diesters

Since the diesters could be hydrolyzed (or metabolized) to the parent diacid in a stepwise manner, several monoesters were also prepared (Scheme 4), and evaluated.



Scheme 4. Synthesis of Monoesters

All diesters and some monoesters were evaluated per ora for lipolysis activity using the rat plasma free fatty acid (FFA) screen. The degree of activity is considered as an indication of their oral absorption. The stability of esters showing good FFA activity, both in solution and in plasma, was also evaluated. Compounds that met the criteria for chemical stability and in vivo activity were further studied for hypoglycemic response in the diabetic mouse, and for pharmacokinetics in both rats and cynomolgus monkeys. Results of these investigations are summarized in Tables 1 to 6.

Pharmacology and Metabolism

Rat Plasma Free Fatty Acid (FFA) Screen: Rats are fasted overnight and given drugs orally using 0.5% methylcellulose/0.1% tween-80 as vehicle. Blood is analyzed 1 h after dosing, and elevation of plasma FFA is used as an indicator of β_3-adrenergic activity on white adipose tissue. Drugs are given in a single dose (0.1 mg/kg) at which the parent drug (**CL316243**) does not give any significant plasma FFA elevation. In the FFA assay, **CL317413** (diisopropyl ester) is ten times more potent than **CL316243**, and is used as a standard (its FFA response is set at 100%) for comparison.

Diabetic Mouse Hypoglycemic Response: Diabetic mice are fed drugs mixed in the diet, ad libitum. Drugs are given in a dose–response manner for 5–7 days. The % glucose lowering is calculated along with drug intake over the test period.

Chemical Stability Study: Prodrugs are dissolved in a mixture of 30% acetonitrile and 70% of the appropriate physiological buffer (pH 1, pH 7.4, pH 9) or water. The extent of any degradation is analyzed by HPLC over 23 h at 37 °C.

Metabolic Stability and Pharmacokinetics studies: In vitro stabilities of selected diester prodrugs (**CL317413**, compounds **3**, **5**, and **12**) were studied in rat, monkey, and human plasmas. In vivo PK studies in rats and cynomolgus monkeys were performed on compounds **3** and **5**.

Table 1. Alkyl Esters of CL316243

Compd	R	Rat FFA at 0.1 mg/kg (% CL317413 Response) Diester	Monoester
CL316243	Na	2	
CL317413	i-Propyl	100	45
1	Ethyl	114	57
2	Butyl	83	
3	2,2-Dimethylpropyl	134	81
4	1-Ethylpropyl	63	52
5	3-Methylbutyl	109	42
6	n-Hexyl	51	66

Table 1. (Cont'd)

Compd	R	Rat FFA at 0.1 mg/kg (% CL317413 Response) Diester	Monoester
7	2-Ethylbutyl	86	56
8	3,3-Dimethylbutyl	71	61
9	n-Octyl	46	45
10	Cyclopropylmethyl	99	37
11	2-Cyclopropylethyl	77	19
12	Cyclobutylmethyl	83	39
13	Cyclopentyl	118	
14	Cyclohexyl	59	
15	Cyclohexylmethyl	22	47
16	(1-Methylcyclohexyl)methyl	84	
17	Trimethylsilylmethyl	73	30
18	2-Trimethylsilylethyl	60	30
19	3-Hydroxy-2,2-dimethylpropyl	78	
20	Allyl	86	

Table 2. Arylalkyl Diesters of CL316243

Compd	R	Rat FFA at 0.1 mg/kg (% CL317413 Response)
21	Benzyl	21
22	2-Phenylethyl	95
23	1-Phenylethyl	78
24	3-Phenylpropyl	61
25	4-Phenylbutyl	43
26	5-Phenylpentyl	93
27	2,2-Dimethyl-3-phenylpropyl	25
28	Cinnamyl	58
29	3-Chlorobenzyl	46
30	4-Methoxybenzyl	8
31	2-(3-Methylphenyl)ethyl	39
32	2-(3-Trifluoromethylphenyl)ethyl	28
33	2-(3-Bromophenyl)ethyl	71
34	2-(2-Thienyl)ethyl	77
35	2-(3-Thienyl)ethyl	80

Table 3 Alkoxyalkyl Esters of CL316243

Compd	R	Rat FFA at 0.1 mg/kg (% CL317413 Response) Diester	Monoester
36	2-Methoxyethyl	65	85
37	2-Ethoxyethyl	101	73
38	2-i-Propoxyethyl	86	
39	2-Butoxyethyl	58	83
40	2-i-Butoxyethyl	63	
41	2-t-Butoxyehtyl	14	20
42	2-Phenoxyethyl	2	51
43	2-Benzyloxyethyl	120	109
44	3-Benzyloxypropyl	83	
45	2-(4-Chlorophenoxy)ethyl	26	
46	2-(3-Chlorophenoxy)ethyl	88	
47	2-(2-Methoxyethyoxy)ethyl	71	
48	Tetrahydrofurfuryl	24	
49	Tetrahydrofuran-3-ylmethyl	68	
50	Tetrahydropyran-2-ylmethyl	11	

Table 4. Acylmethyl Diesters of CL316243 [R = $CH(R_1)COR_2$]

Compd	R_1	R_2	Rat FFA at 0.1 mg/kg (% CL317413 Response)
51	H	Phenyl	7
52	Me	Methoxy	42
53	H	Ethoxy	22
54	Me	Ethoxy	9
55	H	n-Propoxy	30
56	H	i-Propoxy	78
57	H	Phenoxy	37

Table 5. (Acyloxy)alkyl Diesters of CL316243 [R = $CH(R_1)O(COR_2)$]

Compd	R_1	R_2	Rat FFA at 0.1 mg/kg (% CL317413 Response)
58	H	Methyl	39
59	H	Ethyl	10
60	Me	Ethyl	2
61	H	Propyl	24
62	H	i-Propyl	26
63	H	t-Butyl	14
64	Me	t-Butyl	23
65	H	3-Methylbutyl	37
66	H	n-Hexyl	23
67	H	Cyclohexyl	19
68	H	Phenyl	31
69	Me	Phenyl	30
70	Me	Cyclohexyloxy	17

Table 6. Dose-Response Study in Rats (FFA) and Diabetic Mice (Glucose), and LogP Data of Selected Compounds

Compd	R	Rat FFA ED_{50} (mg/kg)	db/db Mice Glucose Lowering ED_{50} (mg/kg)	logP*
CL316243	Na	0.240	0.540	0.046
CL317413	i-Propyl	0.061	0.056	1.29
1	Ethyl	0.034	0.100	0.35
3	2,2-Dimethylpropyl	0.027	0.028	2.16
5	3-Methylbutyl	0.026	0.110	3.77
8	3,3-Dimethylbutyl	0.080	0.201	3.21
12	Cyclobutylmethyl	0.071	0.128	2.34
22	2-Phenylethyl	0.071	0.156	na
34	2-(2-Thienyl)ethyl	0.076	0.208	na

*: equilibrium solubility method
na: not measured

Results and Discussion

In vitro stability studies in rat plasma showed fast hydrolysis of the diesters to the parent diacid, with very short half-lives (several minutes) for the monoesters. No measurable esters (di- or mono-) were detected in vivo, following oral administration of the diester. These observations confirm the prodrug nature of the diesters.

All esters evaluated in the rat FFA screen have improved activity over **CL316243**, indicating enhancement of absorption through increased lipophilicity. In general, with a few exceptions, the diester is more potent than the corresponding monoester. Most simple esters (Tables 1, 2, and 3) are more active than the double esters (Tables 4 and 5) which also lack chemical stability.

Diesters are hydrolyzed extensively (presumably to the monoesters) at pH 9, while reasonably stable at neutral or lower pH. The di-neopentylester (compound **3**) shows the best chemical stability, with unchanged material of 95% (water), 85% (pH 7.4), 80% (pH 1), and 40% (pH 9) after 23 h at 37 °C.

Based on stability, rat FFA activity, and physical properties, several diesters were selected for further biological evaluations. Results in Table 6 indicate five to ten fold improvements over **CL316243** in both FFA and glucose lowering screens. In vivo PK studies (in rats) of the two best compounds **3** and **5** show forty and ten fold increases in oral bioavailability respectively.

In monkey or human plasma, the diesters are rapidly hydrolyzed to their monoesters ($t_{1/2}$ = 3–4 min), followed by a slow conversion to the diacid ($t_{1/2}$ > 4 h). Compounds **3** and **5** show two and three fold enhanced oral bioavailability respectively in the PK study in cynomolgus monkeys. (The oral bioavailabilities of **CL316243** in rats and cynomolgus monkeys are 12% and 3% respectively.)

In conclusion, we have studied the chemical and metabolic stabilities, as well as the in vivo effects of various esters of **CL316243**. Some simple alkyl diesters have been found to be viable prodrugs with improved oral bioavailability.

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