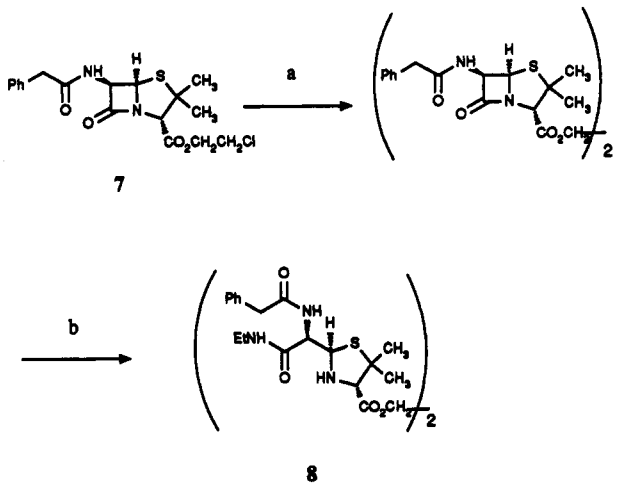
Table I. Inhibition of HIV-1 Proteinase by Penicillin-Derived Dimers and Related Compounds

no.	IC_{50},[a] nM
2	>70000
3a	60
3b	3.0
3c	4.8
3d	0.9
4	500
5a	>12000
5b	6800
6a	>63000
6b	>55000
8	>12000

[a] Assay procedure as in ref 7.

Scheme II[a]



[a] (a) 1 (K salt), DMF, 100 °C for 4 h; (b) $EtNH_2$, CH_2Cl_2 for 24 h.

important for inhibitory activity, but that the amide groups in the linker of **3a** are possibly involved in important H-bonding interactions with the enzyme.

Diester **3a** failed to block the cytopathic effect of HIV-1 in MT-4 cells.[10] However, diamides **3c** and **3d**, both potent inhibitors of the proteinase enzyme, had EC_{50} activities of 5.4 and 0.29 μM, respectively, in HIV-1 infected MT-4 cells. The more hydrophilic diamide **3b** failed to show an antiviral effect possibly because of lack of penetration into the cells. Compounds **3c** and **3d** inhibit syncytia formation of HIV-1 infected C8166 cells at concentrations of 1.11 and 0.06 μM, respectively, and also the expression of p24 core antigen in H9 cells at concentrations of 0.42 and 0.025 μM, respectively.[11] Neither compound exhibited cytotoxicity in any of the cellular assays, nor did they show activity against other aspartyl proteinases (renin, pepsin, or cathepsin D) at concentrations up to 100 μg/mL.

Crystal structures of HIV-1 proteinase both in its native form and complexed with various inhibitors have been described.[6] In view of the activity of the symmetric dimers **3** and the contrasting inactivity of the amides **6**, it was reasonable to speculate that they bind symmetrically at the active site. An X-ray structure[12] of the diamide **3c** complexed to recombinant enzyme[13] has shown that the interaction is indeed symmetrical.

Workers at Abbott Laboratories have designed an elegant series of C_2-symmetric inhibitors of HIV-1 proteinase which also inhibit HIV-1 replication.[14,15] Our compounds also exhibit C_2 symmetry but have the advantage of being less peptidic in character. Studies are in progress to assess the potential of this novel series of compounds as chemotherapeutic agents for the treatment of AIDS.

Acknowledgment. We thank Dr. Stuart B. Laing and Dr. Peter S. Marshall for help with HPLC purifications and Miss Rachel Potter for assistance with the enzyme assays. We are grateful to the Medical Research Council AIDS Reagent Project for supplying the viruses and cells necessary for this study.

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† Department of Virology.
‡ Department of Genetics.

**David C. Humber,* Nicholas Cammack†
Jonathan A. V. Coates,† Kevin N. Cobley, David C. Orr†
Richard Storer, Gordon G. Weingarten
Malcolm P. Weir‡**

*Departments of Medicinal Chemistry II, Virology and Genetics
Glaxo Group Research Limited
Greenford, Middlesex, UB6 0HE, United Kingdom*

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Disodium (*R,R*)-5-[2-[[2-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243). A Potent β-Adrenergic Agonist Virtually Specific for β_3 Receptors. A Promising Antidiabetic and Antiobesity Agent

β-Adrenoceptors have been subclassified as β_1 and β_2 since 1967.[1] Increased heart rate is the primary consequence of β_1-receptor stimulation, while bronchodilation and smooth muscle relaxation typically result from β_2 stimulation. Rat adipocyte lipolysis was initially thought to be a β_1-mediated process.[1] However, more recent results indicate that the receptor-mediating lipolysis is neither β_1 nor β_2, but "atypical" in nature.[2] These "atypical" re-

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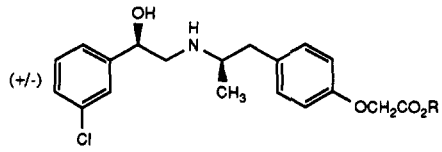
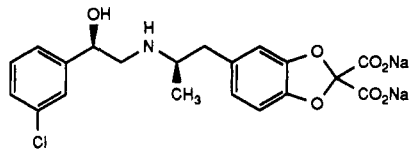


1 R= CH_3; BRL 35,135 (HBr salt)

2 R= H; BRL 37,344

Figure 1.



3 CL 316,243

Figure 2.

ceptors, later called β_3-adrenoceptors,[3] are found on the cell surface of both white and brown adipocytes where their stimulation promotes both lipolysis (breakdown of fat) and energy expenditure.[2]

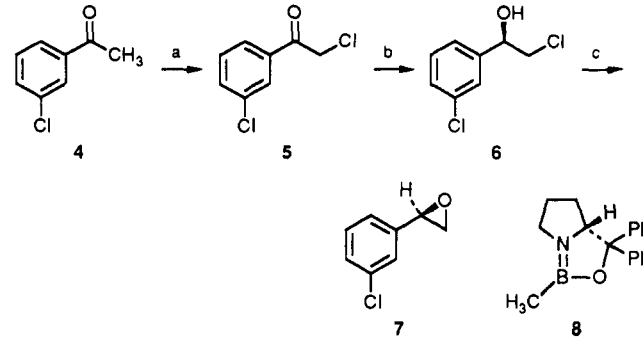
Major support for this third subtype of β-receptor came from a series of phenethanolamines reported by Beecham Laboratories,[4,5] which were found to be more potent agonists for stimulation of lipolysis than for stimulation of atrial rate (β_1) or tracheal relaxation (β_2). The two compounds showing the greatest β_3 selectivity, BRL 35,135 (1) and its active metabolite, BRL 37,344 (2), are shown in Figure 1.

Such selectivity for β_3-adrenoceptors could make compounds of this type potentially useful as antiobesity agents.[4] In addition, these compounds have been reported to show antihyperglycemic effects in animal models of non-insulin-dependent diabetes mellitus.[6,7]

A major drawback in treatment of chronic diseases with β_3 agonists is the potential for stimulation of other β-receptors and subsequent side effects. The most likely of these include muscle tremor (β_2) and increased heart rate (β_1). Although the Beecham compounds do possess β_3 selectivity, mild side effects of this type have been observed in human volunteers.[8,9] It is reasonable to expect that these side effects resulted from partial β_1 and/or β_2 agonism.

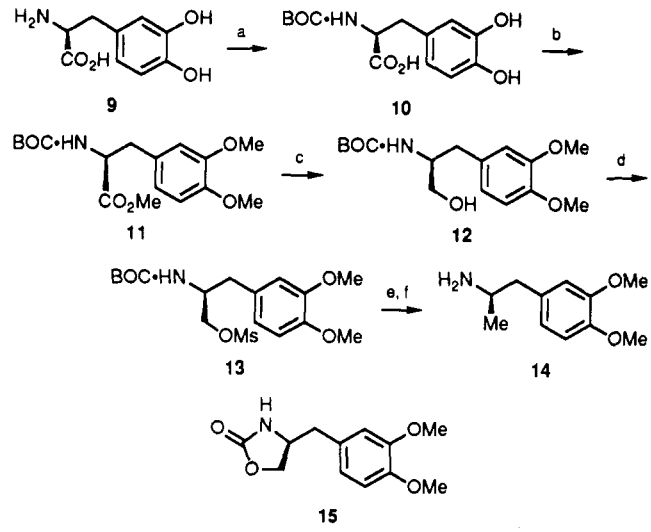
We wish to report the chiral synthesis and biological evaluation of 3 (CL 316,243), a benzodioxole-containing phenethanolamine (Figure 2), which is a highly potent stimulant of adipocyte lipolysis, but is an extremely poor β_1 and β_2 agonist. The β_3 selectivity of this compound is greater than that of any agent previously reported. We believe that 3 offers an exciting possibility for the treatment of non-insulin-dependent diabetes as well as obesity without undesired β-mediated side effects.

Scheme I[a]



[a] Reagents: (a) SO_2Cl_2, 41%; (b) 8, borane–THF, 87%; (c) K_2CO_3, acetone, 89%.

Scheme II[a]



[a] Reagents: (a) di-*tert*-butyl dicarbonate/DMF; (b) MeI, K_2CO_3, 81% for two steps; (c) $LiBH_4$, 98%; (d) MsCl, TEA, 86%; (e) TFA; (f) H_2/Pd–C, 52% for two steps.

Chemistry

Our desire to evaluate 3 as the single active[10] enantiomer (*R,R*) required that two chiral fragments, epoxide 7 and amine 14, be prepared in high optical purity. Thus, monochlorination of *m*-chloroacetophenone (4) (Scheme I) yielded the crystalline phenacyl chloride 5.[11] Asymmetric reduction of 5 using Corey's CBS reagent[12] (8) derived from

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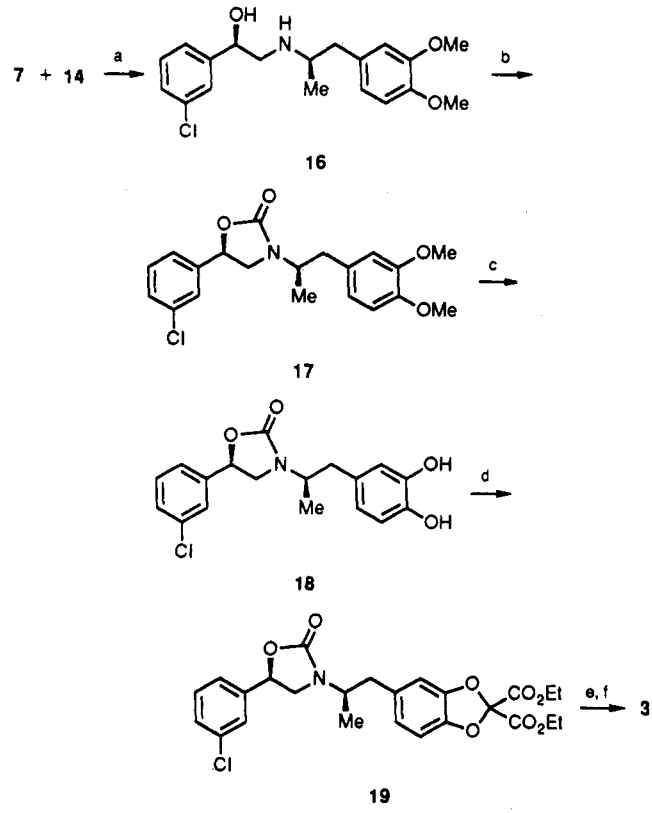
(10) Initially, racemic 3 was prepared by a different route. This material was resolved via the Mosher amide (see ref 13) and both diastereomeric amides were reconverted to the free amine by basic hydrolysis. Virtually all lipolytic activity resided in the (–)-*R,R* enantiomer. This is consistent with data published by Beecham. See: Cantello, B. C. C.; Smith, S. A. BRL 35135. *Drugs Future* **1991**, *16*, 797–800. Also see ref 7.

(11) Wornhoff, E. W.; Martin, D. G.; Johnson, W. S. *Organic Synthesis*; Wiley: New York, 1963; Collect. Vol. IV, pp 162–166.

Scheme III[a]



[a] Reagents: (a) TMS acetamide/DMSO; (b) carbonyldiimidazole, then crystallize, 57% for two steps; (c) BBr_3, 90%; (d) $EtO_2CC(Br)_2CO_2Et$, K_2CO_3, 64%; (e) NaOH, heat; (f) C_{18} column, 85%.

D-proline gave the chiral chlorohydrin **6** in high yield, which had an ee of 85% as determined by NMR analysis of the Mosher ester.[13] The epoxide **7** was readily formed by treating **6** with K_2CO_3 in refluxing acetone.

Preparation of the required chiral amine **14** (Scheme II) began with BOC protection of L-DOPA (**9**), followed by permethylation to give the ester **11**. Lithium borohydride reduction yielded the primary alcohol **12**, which was treated with methanesulfonyl chloride to provide the rather unstable mesylate **13**. The mesylate converted to urethane **15** upon standing; however, immediate removal of the BOC group followed by hydrogenation of the resulting trifluoroacetate salt gave pure 3,4-dimethoxyamphetamine (**14**). The proton NMR spectrum of the Mosher amide[13] of **14** revealed only a single isomer.

The opening of epoxide **7** with amine **14**, mediated by *N*-(trimethylsilyl)acetamide,[14] yielded the phenethanolamine **16** contaminated by about 7% of the undesired (*S,R*) diastereomer resulting from the less than optimal ee of **6** (Scheme III). This byproduct was easily removed by conversion to the cyclic urethane **17** and crystallization. Demethylation of **17** followed by bis-alkylation using diethyl dibromomalonate afforded the benzodioxole **19**. Basic hydrolysis cleaved the ester and the urethane groups. The disodium salt **3** was isolated as an amorphous powder by reverse-phase column chromatography.

Table I

agonist	β-adrenergic activities		
	β_3[a,b]	β_1[c,b]	β_2[d,e]
isoproterenol	$(1.3 \pm 0.8) \times 10^{-8}$ (1)	$(1.5 \pm 0.2) \times 10^{-9}$ (1)	$(5.3 \pm 0.3) \times 10^{-9}$ (1)
3	$(3.0 \pm 0.3) \times 10^{-9}$ (1)	$>10^{-4}$ (0.10)[f]	$(3.0 \pm 1.0) \times 10^{-5}$ (0.59)[g]
BRL 37,344	$(8.4 \pm 1.3) \times 10^{-9}$ (1)	$(5.6 \pm 3.3) \times 10^{-6}$ (0.94)	$(1.9 \pm 0.7) \times 10^{-7}$ (0.92)
BRL 35,135	$(1.6 \pm 2.0) \times 10^{-8}$ (0.87)	ND	$(5.0 \pm 3.5) \times 10^{-7}$ (1)

[a] Stimulation of glycerol release from adipocytes from rat epididymal fat pads; see ref 5 for experimental details. [b] Molar EC_{50} values expressed relative to the agonist's own maximal activity; number in parentheses is intrinsic activity relative to that of isoproterenol. [c] Stimulation of the rate of contraction of guinea pig atria: see ref 5 for experimental details. [d] Inhibition of insulin-stimulated [^{14}C]glucose incorporation into glycogen in isolated rat soleus muscle; see ref 17 for experimental details. [e] Molar IC_{50} values expressed relative to the agonist's own maximal activity; number in parentheses is intrinsic activity relative to that of isoproterenol. [f] 10% of maximum isoproterenol activity at 1 mM. [g] 59% of maximum isoproterenol activity at 1 mM. ND, not determined. Values represent the mean ± SEM of 3–17 preparations.

Table II

agonist	binding IC_{50}, M[a]	
	heart (β_1)	soleus (β_2)
isoproterenol	$(6.0 \pm 0.9) \times 10^{-7}$	$(1.0 \pm 0.4) \times 10^{-6}$
3	$(1.3 \pm 0.1) \times 10^{-3}$	$(2.7 \pm 0.7) \times 10^{-4}$
BRL 37,344	$(3.3 \pm 1.3) \times 10^{-6}$	$(1.3 \pm 0.3) \times 10^{-6}$
BRL 35,135	$(5.9 \pm 1.5) \times 10^{-8}$	$(3.0 \pm 0.7) \times 10^{-8}$

[a] Binding affinities; binding was carried out using membranes from rat heart and rat soleus muscle by the method described in ref 18 with the following exceptions: the incubation volume was 0.5 mL and the incubation time was 1 h; the radioligand was [^{125}I]iodocyanopindolol; (−)-isoproterenol (50 μM) was used to define specific binding; the filters were washed at 4 °C. Values are the mean ± SEM of three to six experiments.

Results and Discussion

The in vitro effects of **3** on β_1-, β_2-, and β_3-adrenoceptor mediated processes are summarized in Table I. The compound was a potent stimulant of rat adipocyte lipolysis (β_3 effect), had no effect on the rate of contraction of guinea pig atria (β_1 effect), and had only a very limited ability to inhibit insulin-stimulated [^{14}C]glucose incorporation into glycogen in isolated rat soleus muscle (β_2 effect). In contrast, BRL 37,344 was slightly less potent than **3** in lipolysis, but had considerably more β_1- and β_2-adrenoceptor mediated activities. BRL 35,135 was less potent in lipolysis than BRL 37,344, but had the same β_2 activity. The effects on atrial rate were not investigated. Isoproterenol, a pure β-agonist that is nonselective for β_1- or β_2-adrenoceptors, was used as a reference standard and was a less potent stimulant of lipolysis than of the responses mediated by β_1- or β_2-adrenoceptors.[5]

The ability of the compounds to bind to β-adrenoceptors in membranes from rat heart (β_1-subtype) and rat soleus muscle (β_2-subtype) was determined (Table II). Consistent with the results on activity in Table I, **3** had much lower affinity for both receptors than did either of the Beecham compounds or isoproterenol. The latter com-

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pounds bound equally well to both receptors with the order to potency being BRL 35,135 > BRL 37,344 ≥ isoproterenol. The binding affinities for β_3-adrenoceptors could not be determined since this subtype represents only a small percentage of the adipocyte β-adrenoceptors,[15] and the affinity of these receptors for the β-antagonists used as radioligands is 10–100-fold less than for β_1- or β_2-adrenoceptors.[16]

The antidiabetic and antiobesity properties of **3** also were investigated. Obese (ob/ob) mice, which are a model of non-insulin-dependent diabetes mellitus, were treated for 7 weeks with the compound. The mice (44 g) were allowed to feed ad libitum on normal rodent chow to which the compound had been admixed at a concentration (0.001%) that gave a daily intake of 1 mg/kg body weight. Treatment reduced the amount of weight gained from 13.0 ± 0.6 g in the untreated mice to 7.1 ± 0.6 g ($p < 0.01$) even though food consumption was somewhat greater (4.8 g/mouse per day) than in the untreated mice (4.4 g/mouse per day). Within 1 week, the hyperglycemia (303 ± 28 mg/dL) was reduced to the euglycemia (187 ± 8 mg/dL; $p < 0.01$) found in untreated normal lean littermates (176 ± 4 mg/dL), and the euglycemia was maintained for the duration of the experiment. A manuscript describing more fully the in vivo effects of **3** is in preparation.

Conclusion

Functional in vitro assays, as well as binding experiments, have demonstrated that **3** is a highly potent β_3-adrenoceptor agonist, and that it has a very low affinity for β_1 and β_2 receptors. Since the compound has potent antiobesity and antihyperglycemic effects in animal models of obesity and non-insulin-dependent diabetes, we believe it offers the possibility for treatment of these diseases without undesirable β-mediated side effects. The compound is now in Phase I clinical trials.

Acknowledgment. We wish to thank Dr. F. Lai and Ms. A. Cubuzzi for performing the atrial rate measurements, and Ms. H. Meunkel and Ms. D. Flynn for performing the lipolysis measurements. We also wish to thank Dr. J. James, Dr. M. Siegel, Dr. L. O'Brien, and Mr. G. Morton for spectral data, and Dr. J. Medwid and his staff for analytical data.

Supplementary Material Available: Experimental details for compounds **3**, **5–7**, **10–14**, and **16–19** (8 pages). Ordering information is given on any current masthead page.

Jonathan D. Bloom,* Minu D. Dutia
Bernard D. Johnson, Allan Wissner, Michael G. Burns
Elwood E. Largis, Jo Alene Dolan, Thomas H. Claus

Medical Research Division, Lederle Laboratories
American Cyanamid Company
Pearl River, New York 10965

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