



Pergamon

## HUMAN $\beta_3$ ADRENERGIC RECEPTOR AGONISTS CONTAINING CYCLIC UREIDOBENZENESULFONAMIDES

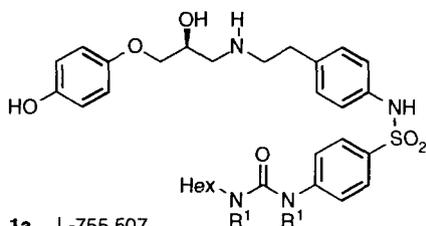
Emma R. Parmee,\* Elizabeth M. Naylor, Leroy Perkins, Vincent J. Colandrea, Hyun O. Ok, Mari R. Candelore, Margaret A. Cascieri, Liping Deng, William P. Feeney, Michael J. Forrest, Gary J. Hom, D. Euan MacIntyre, Randall R. Miller, Ralph A. Stearns, Catherine D. Strader,<sup>1</sup> Laurie Tota, Matthew J. Wyvratt, Michael H. Fisher, and Ann E. Weber

*Departments of Medicinal Chemistry, Molecular Pharmacology/Immunology and Rheumatology, Pharmacology, Drug Metabolism, and Laboratory Animal Resources, Merck Research Laboratories, Rahway, NJ 07065, U.S.A.*

Received 24 November 1998; accepted 28 January 1999

**Abstract:** Human  $\beta_3$  adrenergic receptor agonists containing 5-membered ring ureas were shown to be potent partial agonists with excellent selectivity over  $\beta_1$  and  $\beta_2$  binding. L-760,087 (**4a**) and L-764,646 (**5a**) ( $\beta_3$  EC<sub>50</sub> = 18 and 14 nM, respectively) stimulate lipolysis in rhesus monkeys (ED<sub>50</sub> = 0.2 and 0.1 mg/kg, respectively) with minimal effects on heart rate. Oral absorption in dogs is improved over other urea analogs. © 1999 Elsevier Science Ltd. All rights reserved.

Increasing metabolic rate by activation of the human  $\beta_3$  adrenergic receptor ( $\beta_3$  AR) is an attractive approach toward the treatment of obesity.<sup>2</sup> We have recently reported several series of selective human  $\beta_3$  AR agonists containing a benzenesulfonamide moiety, for example phenol **1a** and pyridines **2**.<sup>3</sup> In the phenolic series, L-755,507 (**1a**) is one of the most potent and selective  $\beta_3$  AR agonists reported to date with a  $\beta_3$  EC<sub>50</sub> value of 0.43 nM, and over 400-fold selectivity over binding to or activation of the  $\beta_1$  and  $\beta_2$  ARs.<sup>3b</sup> This compound has also been shown to increase metabolic rate by 30% with minimal effects on heart rate when administered intravenously at a dose of 0.1 mg/kg to anesthetized rhesus monkeys.<sup>4</sup> The highly polar urea moiety, however, is detrimental to oral absorption, and also, the phenolic derivatives have been shown to undergo substantial *in vivo* glucuronidation.<sup>3c</sup> Cyclic derivatives of L-755,507 (**1b** and **1c**) were prepared in an attempt to reduce the polarity of the molecule whilst maintaining the activity conferred by the urea.<sup>5a</sup> The 6-membered ring analog **1b** was nearly 20-fold less potent than L-755,507 ( $\beta_3$  EC<sub>50</sub> = 8.3 nM); however, the imidazolidinone **1c** was only four-fold less potent than the parent urea ( $\beta_3$  = EC<sub>50</sub> 1.7 nM) and more selective over binding to the  $\beta_1$  and  $\beta_2$  ARs than the 6-membered ring analog (>80-fold selective vs 20-fold).<sup>6,7</sup>

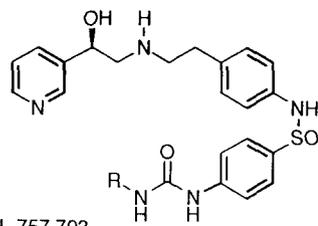


**1a** L-755,507

[R<sup>1</sup> = H]

**1b** [R<sup>1</sup> = -(CH<sub>2</sub>)<sub>3</sub>-]

**1c** [R<sup>1</sup> = -(CH<sub>2</sub>)<sub>2</sub>-]



**2a** L-757,793

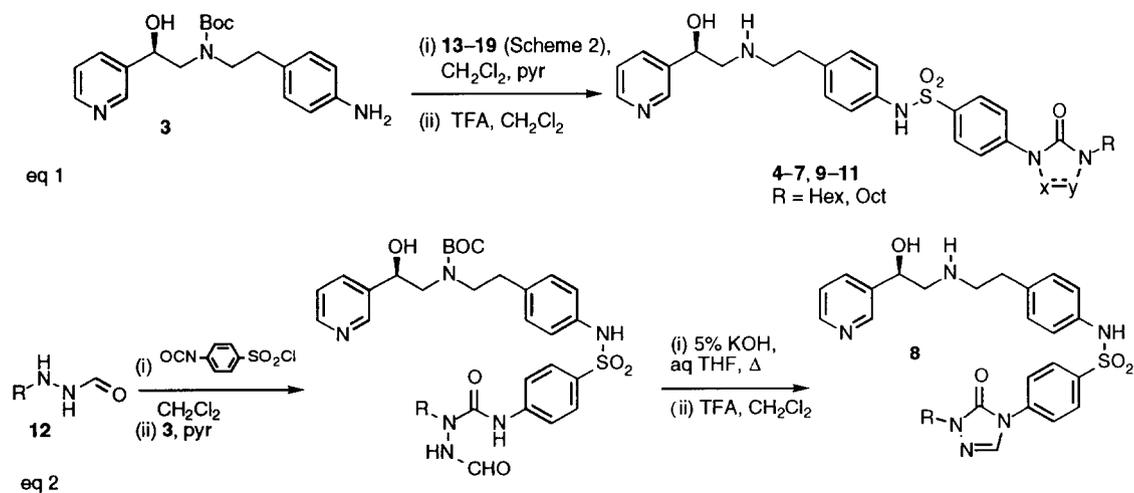
[R = Hex]

**2b** [R = Oct]

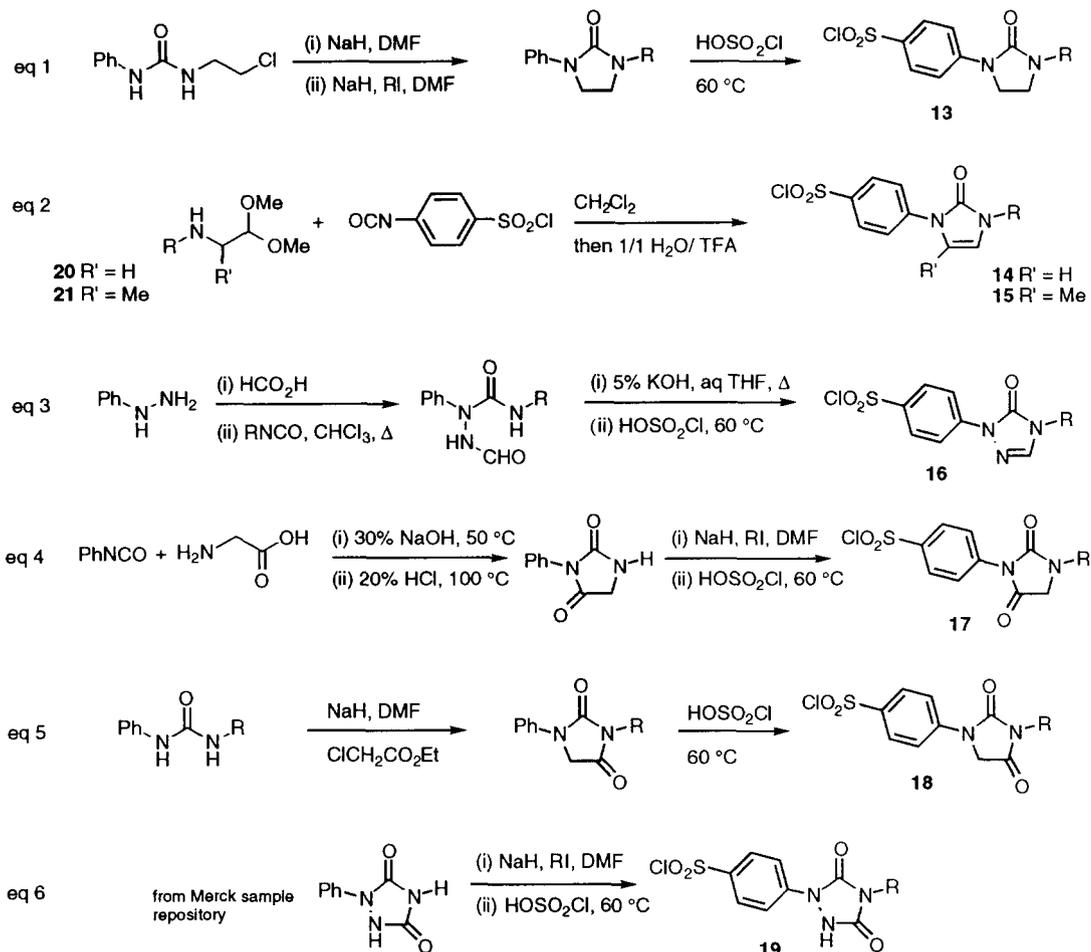
Unlike the phenolic derivatives, the pyridylethanolamines **2** are not prone to glucuronidation. These compounds are selective for the  $\beta_3$  AR over the  $\beta_1$  and  $\beta_2$  ARs, but the ureidobenzenesulfonamide is crucial for  $\beta_3$  potency. The hexyl and octyl analogs **2a** and **2b**, for example, have  $EC_{50}$  values of 6.3 nM and 1.4 nM, respectively.<sup>3d</sup> Oral absorption of the urea containing derivatives was again negligible.<sup>3d</sup> As in the phenol series, we wished to reduce the polarity of these compounds and in this paper we describe the preparation of the more potent imidazolidinones in the (*R*)-pyridylethanolamine series. The study was also extended to include other 5-membered ring urea analogs. As the straight chain hexyl and octyl ureas were among the most potent and selective derivatives, the initial investigation focused only on these two side chains.<sup>3d</sup> Compounds containing a variety of cyclic ureidobenzenesulfonamides were prepared and tested in our cloned human  $\beta$  AR assays.<sup>6,7</sup>

Eight different cyclic urea analogs were chosen [Table 1]; their preparation is shown in Scheme 1.<sup>5</sup> For imidazolidinones **4**, imidazolones **5** and **6**, triazolones **7**, hydantoin **9** and **10**, and triazolidinones **11**, aniline **3** was coupled with sulfonyl chlorides **13–19** and deprotected with trifluoroacetic acid to yield the desired products [Scheme 1, eq 1].<sup>3d,5</sup> For 2-alkyl-4-phenyl triazolones **8** [Scheme 1, eq 2)], formyl hydrazine **12** was added to 4-(chlorosulfonyl)phenylisocyanate followed by immediate treatment with aniline **3**. Cyclization was then effected with potassium hydroxide which, following deprotection, gave the desired products. Preparation of sulfonyl chlorides **13–19** is shown in Scheme 2, and generally involved treatment of the phenyl substituted heterocyclic rings with chlorosulfonic acid. Sulfonyl chlorides **14** and **15** could not be prepared by this method as the imidazolone ring was not stable to chlorosulfonylation conditions, and so amines **20** and **21** were prepared and added to 4-(chlorosulfonyl)phenylisocyanate [Scheme 2, eq 2)]. Subsequent addition of aqueous trifluoroacetic acid to the reaction effected deprotection and in situ cyclization.

**Scheme 1.** Synthesis of  $\beta_3$  AR agonists **4–11** (see Table 1) containing cyclic ureidobenzenesulfonamides.

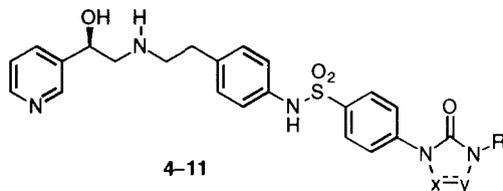


## Scheme 2. Synthesis of sulfonyl chlorides 13–19.



All the analogs were tested at the human  $\beta$  ARs; the *in vitro* results are shown in Table 1.<sup>6,7</sup> Derivatives **4–10** and **11b** were partial to full agonists of the  $\beta_3$  receptor (44–100% activation). Hexyl triazolidinone **11a** did not activate the  $\beta_3$  AR at 100 nM, and hence did not meet our criteria for titration to determine the  $EC_{50}$ . As with the pyridylethanolamine urea derivatives **2**, the longer octyl side chain generally gave significantly more potent compounds, although this did not necessarily result in a higher degree of selectivity over binding to the  $\beta_1$  and  $\beta_2$  ARs. The hexyl analogs showed no agonist activity at the  $\beta_1$  and  $\beta_2$  ARs at 10  $\mu$ M. The octyl analogs were, however, weak partial agonists at the  $\beta_1$  receptor (< 30% activation at 10  $\mu$ M, data not shown).

Octyl imidazolidinone **4b** was the most potent 5-membered ring derivative prepared in the pyridylethanolamine series ( $\beta_3$   $EC_{50}$  = 2.2 nM), and although the hexyl derivative **4a** was more than eight-fold less potent, both compounds exhibited a similar degree of selectivity over binding to the  $\beta_1$  and  $\beta_2$  ARs (>125-fold selective). The unsaturated analogs, imidazolones **5a** and **5b**, showed a very similar trend in potency with the longer chain again resulting in a more potent compound. In this series, however, the hexyl derivative, L-

**Table 1.** Activity of derivatives **4–11** containing cyclic ureidobenzenesulfonamides at the cloned human  $\beta$  adrenergic receptors.

Compound	R <sup>a</sup>	x	y	nM $\beta_3$ EC <sub>50</sub> (% act) <sup>b</sup>	$\beta_1$ binding IC <sub>50</sub> <sup>c</sup> (nM)	$\beta_2$ binding IC <sub>50</sub> <sup>c</sup> (nM)
<b>4a</b>	Hex	CH <sub>2</sub>	CH <sub>2</sub>	18 (62)	5000	2300
<b>4b</b>	Oct	CH <sub>2</sub>	CH <sub>2</sub>	2.2 (62)	580	380
<b>5a</b>	Hex	CH	CH	14 (56)	18000	12000
<b>5b</b>	Oct	CH	CH	3.4 (63)	5500	330
<b>6a</b>	Hex	CH	CMe	81 (100)	2000	7000
<b>6b</b>	Oct	CH	CMe	60 (100)	2000	7000
<b>7a</b>	Hex	N	CH	6 (56)	8500	4500
<b>7b</b>	Oct	N	CH	5.4 (68)	850	730
<b>8a</b>	Hex	CH	N	100 (44)	>10000	8500
<b>8b</b>	Oct	CH	N	15 (82)	2000	2000
<b>9a</b>	Hex	C=O	CH <sub>2</sub>	130 (47)	>10000	>10000
<b>9b</b>	Oct	C=O	CH <sub>2</sub>	16 (64)	5000	5000
<b>10a</b>	Hex	CH <sub>2</sub>	C=O	13 (70)	7000	2000
<b>10b</b>	Oct	CH <sub>2</sub>	C=O	4.9 (65)	2500	370
<b>11a</b>	Hex	N	C=O	(7) <sup>d</sup>	10000	10000
<b>11b</b>	Oct	N	C=O	100 (63)	10000	3000

<sup>a</sup>Hexyl and octyl chains were unbranched for all examples. <sup>b</sup>Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol. <sup>c</sup>Receptor binding assays were carried out with membranes prepared from CHO cells expressing the cloned human receptor in the presence of <sup>125</sup>I-iodocyanopindolol. <sup>d</sup>Single point data, % activation at 100 nM.

764,646 (**5a**), was over 850-fold selective for agonist activity at the  $\beta_3$  AR over binding to and activation of the other  $\beta$  ARs. L-764,646 binds to the  $\beta_3$  AR with an IC<sub>50</sub> value of 81 nM and exhibited excellent selectivity (>100-fold) for agonist activity over a wide range of other receptors which were assayed.

Installation of a methyl substituent into the 4-position of the imidazolone ring resulted in the only compounds (**6a** and **6b**) which gave 100% activation of the  $\beta_3$  AR. There was, however, a significant loss in potency ( $\beta_3$  EC<sub>50</sub> = 60–80 nM). Insertion of a nitrogen atom into the imidazolone ring gave triazolones **7** and **8**. The position of the hetero atom was important to maintain potency at the  $\beta_3$  AR. Both 4-alkyl-2-phenyl-triazolone derivatives **7a** and **7b** were potent partial agonists of the  $\beta_3$  AR. Hexyl derivative **7a** also showed excellent selectivity over binding at the  $\beta_1$  and  $\beta_2$  ARs (1400-fold and 750-fold, respectively). The alternate isomers **8a** and **8b** were significantly less potent  $\beta_3$  agonists.

The position of the carbonyl group was also found to be important when the two series of hydantoin **9** and **10** were tested. When the substitution was made in the 4-position (**10a** and **10b**) there was very little difference in potency or selectivity between these two derivatives and the imidazolidinones **4** and imidazolones **5**; octyl derivative **10b** was the most potent ( $\beta_3$  EC<sub>50</sub> = 4.9 nM), however, the hexyl analog **10a** was more selective ( $\beta_3$  EC<sub>50</sub> = 13 nM, > 160-fold selective over binding at the  $\beta_1$  and  $\beta_2$  ARs). The isomeric hydantoin **9a** and **9b** were several times less potent at the  $\beta_3$  AR than the unsubstituted ring systems, although the octyl analog **9b** did show > 330-fold selectivity over binding to the  $\beta_1$  and  $\beta_2$  ARs. Triazolidinones **11** contained both the nitrogen atom and the carbonyl group in the position which led to the most potent triazolone **7** and hydantoin **10**. The effect was not additive as the hexyl analog did not activate the  $\beta_3$  AR at 100 nM and the longer chain derivative was of modest potency.

Three derivatives (**4a**, **4b**, and **5a**) were administered intravenously in a rising dose study to anesthetized rhesus monkeys.<sup>8</sup> Hexyl and octyl imidazolidinones **4a** and **4b** show an eight-fold difference in in vitro potency ( $\beta_3$  EC<sub>50</sub> = 18 and 2.2 nM, respectively); however, both compounds exhibited a nearly full lipolytic response in vivo with a maximum response 80% of that of isoproterenol and similar ED<sub>50</sub> values for glycerolemia (0.2 and 0.1 mg/kg, respectively). Minimal heart rate increases were seen at 10 mg/kg ( $\leq 15\%$ ). L-764,646 (**5a**) had a maximum response which was 60% of that of isoproterenol with an ED<sub>50</sub> value for glycerolemia of 0.1 mg/kg. This compound showed enhanced selectivity as significant heart rate effects were not seen until a dose of 30 mg/kg was administered.

To establish if these less polar cyclic compounds would have improved absorption, the oral bioavailabilities of two representative compounds, L-760,087 (**4a**) and L-764,646 (**5a**), were determined in dogs (3 mg/kg iv, 10 mg/kg po). Both L-760,087 and L-764,646 had oral bioavailabilities of 7% (PEG/EtOH/saline vehicle) with excellent half-lives (20 and 17 h, respectively). While the bioavailabilities were still relatively low, radiolabelling experiments suggested that removing the highly polar urea had, as hoped, greatly improved absorption. Thus, administration of [<sup>3</sup>H]-L-760,087 to bile duct cannulated dogs at a dose of 10 mg/kg led to an overall absorption of radioactivity of 29% (12% recovered in the bile and 17% recovered in the urine).<sup>9</sup>

In conclusion, we have identified several new series of human  $\beta_3$  adrenergic receptor agonists which have good potency and selectivity but do not contain the highly polar urea moiety. L-764,646 (**5a**), in particular, is an exceptionally selective human  $\beta_3$  agonist ( $\beta_3$  EC<sub>50</sub> = 14 nM), with >850-fold selectivity over binding to or activation of the  $\beta_1$  and  $\beta_2$  receptors and >100-fold selectivity over all other receptors assayed. When administered intravenously to rhesus monkeys, imidazolone L-764,646, and members of the imidazolidinone series evoke lipolysis at low doses with minimal effects on heart rate. The oral bioavailability of L-760,087 (**4a**) and L-764,646 (**5a**), though still modest, is a marked improvement over the negligible absorption of parent urea L-757,793 (**2a**). It was subsequently found that slight modification both to the side-chain and cyclic ureidobenzenesulfonamide led to human  $\beta_3$  AR agonists with dramatically improved bioavailabilities. These alterations will be the subject of future publications.

**Acknowledgments:** We thank Mr. Paul Cunningham and Mr. Donald Hora, Jr. for expert technical assistance with the in vivo experiments, Professor James G. Grannemann (Wayne State University) for supplying the cloned human  $\beta_3$  AR, Dr. Yui Tang for preparing [<sup>3</sup>H]-L-760,087, and Ms. Amy Bernick for mass spectrometric analyses.

## References and Notes

1. Present address: Schering Plough Research Institute, 2015 Galloping Hill Rd, Kenilworth, NJ 07033.
2. For recent reviews see: (a) Dow, R. L. *Exp. Opin. Invest. Drugs* **1997**, *6*, 1811. (b) Lowell, B. B.; Flier, J. S. *Annu. Rev. Med.* **1997**, *48*, 307. (c) Arch, J. R. S.; Wilson, S. *Int. J. Obesity* **1996**, *20*, 191. (d) Himmis-Hagen, J.; Danforth, E. *Curr. Opin. Endocrin. Diabetes* **1996**, *3*, 59. (e) Claus, T. H.; Bloom, J. D. *Ann. Rep. Med. Chem.* **1995**, *30*, 189.
3. (a) Weber, A. E.; Mathvink, R. J.; Perkins L.; Hutchins, J. E.; Candelore, M. R.; Tota, L.; Strader, C. D.; Wyvratt, M. J.; Fisher, M. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1101. (b) Parmee, E. R.; Ok, H. O.; Candelore, M. R.; Tota, L.; Deng, L.; Strader, C. D.; Wyvratt, M. J.; Fisher, M. H.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1107. (c) Weber, A. E.; Ok, H. O.; Alvaro, R. F.; Candelore, M. R.; Cascieri, M. A.; Chiu, S-H. L.; Deng, L.; Forrest, M. J.; Hom, G. H.; Hutchins, J. E.; Kao, J.; MacIntyre, D.E.; Mathvink, R. J.; McLoughlin, D.; Miller, R.R., Newbold, R. C.; Olah, T. V.; Parmee, E. R.; Perkins L.; Stearns, R. A.; Strader, C.D.; Szumiloski, J.; Tang, Y.S.; Tota, L.; Vicario, P. P.; Wyvratt, M. J.; Fisher, M. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2111. (d) Naylor, E. M.; Colandrea, V. J.; Candelore, M. R.; Cascieri, M. A.; Colwell, L. F.; Deng, L.; Feeney, W. P.; Forrest, M. J.; Hom, G. H.; MacIntyre, D.E.; Strader, C.D.; Tota, L.; Wang, P.-R.; Wyvratt, M. J.; Fisher, M. H.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3087.
4. Fisher, M. H.; Amend, A. M.; Bach, T. J.; Barker, J. M.; Brady, E. J.; Candelore, M. R.; Carroll, D.; Cascieri, M. A.; Chiu, S-H. L.; Deng, L.; Forrest, M. J.; Hegarty-Friscino, B.; Guan, X.-M.; Hom, G. H.; Hutchins, J. E.; Kelly, L. J.; Mathvink, R. J.; Metzger, J. M.; Miller, R. R.; Ok, H.O.; Parmee, E. R.; Saperstein, R.; Strader, C. D.; Stearns, R. A.; Thompson, G. M.; Tota, L.; Vicario, P. P.; Weber, A. E.; Woods, J. W.; Wyvratt, M. J.; Zafian, P. T.; MacIntyre, D. E. *J. Clin. Invest.* **1998**, *101*, 2387.
5. (a) All compounds were characterized by <sup>1</sup>H NMR, mass spectrometry, and HPLC analysis prior to submission for biological evaluation. For experimental details see: Fisher, M. H.; Mathvink, R. J.; Ok, H.O.; Parmee, E. R.; Weber, A. E. U. S. Patent 5 451 677, 1995; *Chem. Abstr.* **1996**, *124*, 116877 and Fisher, M. H.; Naylor, E. M.; Weber, A. E. U. S. Patent 5 541 197, 1996; *Chem. Abstr.* **1996**, *125*, 221588. (b) The 3-pyridylethanolamines **4–11** were prepared as the optically active (*R*)-enantiomers from aniline **3** (90% ee).<sup>3d</sup> Several pairs of (*R*)- and (*S*)-enantiomers in this 3-pyridylethanolamine series have been synthesized and their β<sub>3</sub> AR agonist activity examined. In each case, in line with expectation, the (*R*)-isomer was 5- to 190-fold more potent than the respective (*S*)-isomer.
6. The human β<sub>3</sub> AR was obtained from Professor J. Grannemann (Wayne State University), Granneman, J. G.; Lahners, K. N.; Rao, D. D. *Mol. Pharmacol.* **1992**, *42*, 964. The human β<sub>1</sub> and β<sub>2</sub> ARs were cloned as described in Frielle, T.; Collins, S.; Daniel, K. W.; Caron, M. G.; Lefkowitz, R. J.; Kobilka, B. K. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7920 and Kobilka, B. K.; Dixon, R. A.; Frielle, T.; Dohlman, H. G.; Bolanoski, M. A.; Sigal, I. S.; Yan-Feng, T. L.; Francke, U.; Caron, M. G.; Lefkowitz, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 46. The receptors were expressed in CHO cells at receptor densities of 46–88 fmol/mg (β<sub>3</sub> receptors) or 300–500 fmol/mg (β<sub>1</sub> and β<sub>2</sub> ARs). Agonist activity and binding affinity were assessed by measurement of cellular cAMP levels relative to isoproterenol and inhibition of <sup>125</sup>I-cyanopindolol binding, respectively.
7. Compounds were assayed for their ability to stimulate increases in cAMP in CHO cells expressing the cloned human β<sub>3</sub> AR. The activity of an agonist at the β<sub>3</sub> AR is best described by its ability to stimulate adenylyl cyclase in a functional assay since this method measures affinity for the high affinity, G-protein coupled state of the receptor. This accurately predicts the lipolytic potential of compounds in native adipocytes.<sup>4</sup> The β<sub>3</sub> AR IC<sub>50</sub> values are a measure of the compounds binding affinity for both the high and low affinity states of the β<sub>3</sub> AR, thus are lower than the respective EC<sub>50</sub> values. These derivatives exhibited very low efficacy at the β<sub>1</sub> and β<sub>2</sub> ARs (<30% activation at 10 μM), hence the selectivity of the compounds is most accurately represented by comparing the β<sub>3</sub> EC<sub>50</sub> values with the β<sub>1</sub> and β<sub>2</sub> IC<sub>50</sub> values.
8. All in vivo experiments were performed as described in ref 4.
9. [<sup>3</sup>H]-L-760,087 was prepared from aniline **3** as described in ref 3c.