Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3087–3092

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

3-PYRIDYLETHANOLAMINES: POTENT AND SELECTIVE HUMAN β3 ADRENERGIC RECEPTOR AGONISTS

Elizabeth M. Naylor,* Vincent J. Colandrea, Mari R. Candelore, Margaret A. Cascieri, Lawrence F. Colwell, Jr., Liping Deng, William P. Feeney, Michael J. Forrest, Gary J. Hom, D. Euan MacIntyre, Catherine D. Strader,[1] Laurie Tota, Pei-Ran Wang, Matthew J. Wyvratt, Michael H. Fisher, and Ann E. Weber

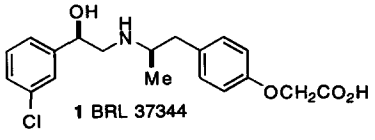
Departments of Medicinal Chemistry, Biochemistry and Physiology, Pharmacology, and Laboratory Animal Resources,

Merck Research Laboratories, Rahway, New Jersey 07065, U.S.A.

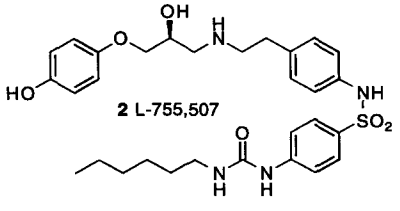
Received 20 July 1998; accepted 16 September 1998

Abstract: The 3-pyridylethanolamine L-757,793 is a potent β_3 AR agonist (EC_{50} 6.3 nM, 70% activation) with 1,300- and 500-fold selectivity over binding to the β_1 and β_2 ARs, respectively. L-757,793 stimulated lipolysis in rhesus monkeys (ED_{50} 0.2 mg/kg) with a maximum response equivalent to that elicited by isoproterenol.

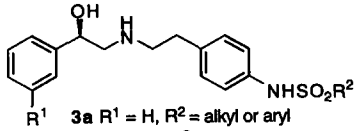
β_3 Adrenergic receptor agonists (AR) are potential anti-obesity drugs.[2] These agents activate specific receptors, located on the surface of adipocytes, causing stimulation of lipolysis and an increase in metabolic rate. A number of different structural classes of β_3 AR agonists have been disclosed.[3] The arylethanolamine BRL 37344 (**1**) was one of the first β_3 AR agonists to be discovered. In rats, BRL 37344 is a potent, selective β_3 AR agonist and was used to identify the β_3 AR in rat brown adipocytes.[4] Subsequently, cloning and expression of the human and rat β_3 ARs indicated differences in their pharmacological properties.[5] In human AR assays performed here at Merck, BR 37344 is a weak β_3 partial agonist (β_3 EC_{50} 450 nM, 23% activation) with β_1 and β_2 AR binding affinities of 5,000 and 3,000 nM, respectively.[6]



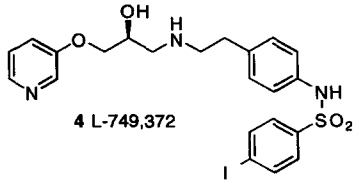
1 BRL 37344



2 L-755,507



3a R^1 = H, R^2 = alkyl or aryl
3b R^1 = Cl, R^2 = alkyl or aryl



4 L-749,372

Recent publications from Merck describe a number of aryloxypropanolamines.[7–10] Aryloxypropanolamine L-755,507 (**2**) is a highly potent human β_3 AR agonist (β_3 EC_{50} 0.43 nM, 52% activation) with greater

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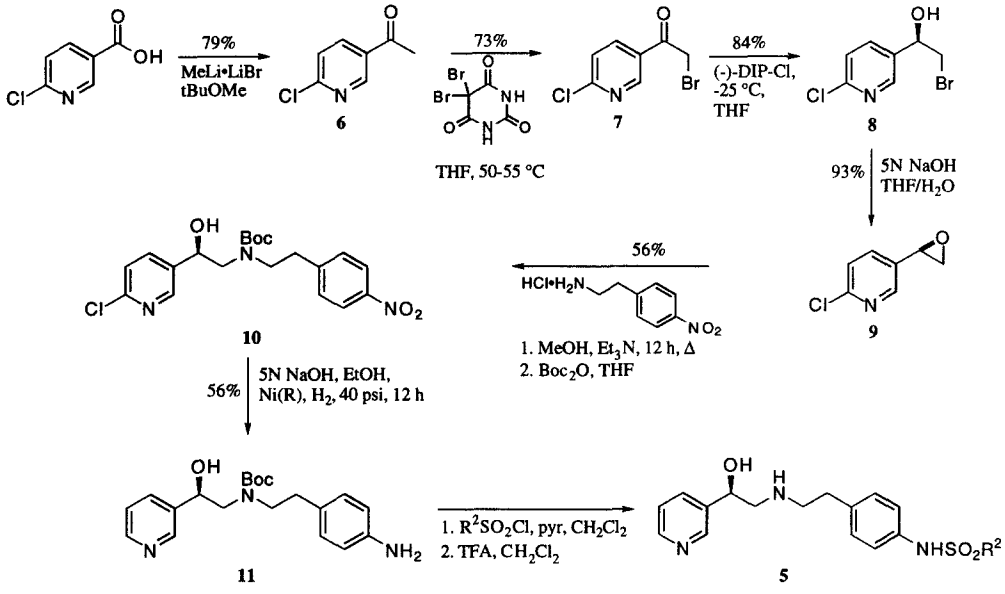
than 400-fold selectivity over binding to the β_1 and β_2 ARs, respectively.[8,9] The sulfonamide moiety present in L-755,507 and related compounds imparts significant potency and selectivity for the human β_3 AR.[7] We decided to investigate whether incorporation of a sulfonamide moiety into the arylethanolamines would have a similar effect.[11]

Series of phenethanolamines **3a** and the 3-chloro analogs **3b** were synthesized and their biological profiles examined.[12] These compounds exhibited minimal β_3 AR agonist activity (data not shown). Modification of the aryloxy group present in the L-755,507 series led to the discovery of 3-pyridyloxypropanolamine L-749,372 (**4**).[10] L-749,372 is a β_3 AR partial agonist (EC_{50} 3.6 nM, 33% activation) with 270- and 30-fold selectivity over binding to the β_1 and β_2 ARs, respectively. In view of the biological profile of L-749,372, we prepared a series of 3-pyridylethanolamines **5**. The synthesis of these compounds is illustrated in the Scheme.[13]

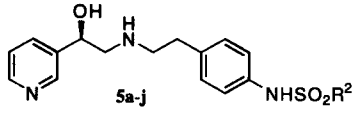
Scheme. Asymmetric Synthesis of 3-Pyridylethanolamines



Reaction of 6-chloronicotinic acid with methyllithium lithium bromide complex gave the methyl ketone **6**.[14] The methyl ketone **6** was treated with bromobarbituric acid in refluxing tetrahydrofuran (THF) to afford the bromoketone **7**. In the absence of the chloro substituent, the bromoketone was unstable. Asymmetric reduction with (-)-DIP-Chloride™ [(-)-*B*-chlorodiisopinocampheylborane] provided the bromohydrin **8** that was converted to the epoxide **9** with base.[15] Epoxide opening with *p*-nitrophenethylamine hydrochloride followed by protection of the resultant secondary amine with di-*tert*-butyldicarbonate gave the protected ethanolamine **10**. Epoxide opening with *p*-nitrophenethylamine rather than *p*-aminophenethylamine produced an improved yield and precluded a selective protection step. Concomitant dechlorination and reduction of the nitrobenzene to aniline were effected by hydrogenation under basic conditions using Raney® nickel as catalyst. Aniline **11** was sulfonylated with the appropriate sulfonyl chloride[16] then deprotected with trifluoroacetic acid

(TFA) to afford the desired 3-pyridylethanolamines **5**. In vitro data for these compounds is shown in Tables 1 and 2.

Table 1. Comparison of the β_3 AR Agonist Activity and β_1 and β_2 Binding Affinity for Sulfonamides **5a–j**



Compound	R^2	β_3 EC_{50}, nM (%act)[a]	β_1 Binding IC_{50}, nM[b]	β_2 Binding IC_{50}, nM[b]
5a	Me	(1)[c]	7,000	10,000
5b	isoPro	(1)[c]	100,000	50,000
5c	$(CH_2)_2Ph$	(12)[c]	100,000	8,000
5d	Ph	160 (55)	20,000	8,000
5e	2-Naphthyl	38 (78)	10,000	1,000
5f	3-Quinolinyl	(26)[c]	100,000	1,000
5g	4-isoPro-Ph	56 (65)	30,000	6,000
5h	4-Cl-Ph	49 (70)	30,000	10,000
5i	4-Br-Ph	77 (65)	10,000	5,000
5j	4-I-Ph	56 (86)	10,000	7,000

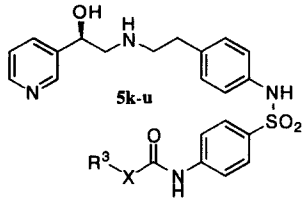
[a]Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol; single point data are reported in parentheses as (% activation @ concentration in nM). [b]Receptor binding assays were carried out with membranes prepared from CHO cells expressing the cloned human receptor in the presence of ^{125}I-iodocyanopindolol. [c]Single point data, % activation at 100 nM.

The methyl-, isopropyl-, and phenethylsulfonamides, **5a**, **5b**, and **5c**, respectively, were essentially inactive in the human β_3 AR assay; however, the benzenesulfonamide **5d** exhibited modest potency and efficacy. Replacement of the phenyl group by a 2-naphthyl moiety led to a 4-fold improvement in β_3 potency. A significant loss in activity was observed with the 3-quinolinylsulfonamide **5f**. Earlier SAR studies in the phenoxypropanolamine series indicated that substitution at the para position of the phenyl ring over the ortho and meta sites was generally the most preferred for β_3 potency enhancement.[7] A number of 4-substituted benzenesulfonamides were prepared and tested. The 4-halo- and 4-isopropylbenzenesulfonamides **5g–j** were all 2- to 3-fold more potent than the parent compound **5d**. These 4-substituted benzenesulfonamides were all at least 100-fold selective (with the exception of the 4-bromo analog, 65-fold selective over β_2 binding) for β_3 AR agonist potency over β_1 and β_2 binding affinity. The ability of these compounds to activate the β_1 and β_2 ARs was low (less than 35% activation at 10 μM).

Next we examined a series of 4-ureidobenzenesulfonamides **5k–o** (Table 2). Increasing the length of the alkyl chain led to enhanced β_3 AR agonist potency. The phenethyl analog **5o** (β_3 EC_{50} 1.6 nM, 55% activation) was equipotent with the octyl urea **5n**. The analogous carbamates **5p–r** and amides **5s–u** were synthesized and tested. In all cases, these compounds were found to have β_3 EC_{50} values less than or equal to their respective urea analogs; however, potencies were usually enhanced with increasingly lipophilic groups as was observed in the urea series. The ureas and amides generally exhibited good selectivity for β_3 AR agonist activity over

binding to the β_1 and β_2 ARs. The carbamates were less selective. In particular, the *n*-hexyl- and phenethylcarbamates **5q** and **5r** were only 14- and 12-fold selective over binding to the β_2 AR. The β_1 and β_2 AR efficacies for these ureas, carbamates and amides were low. The hexylurea **5m** was a weak partial agonist for the β_1 AR (EC_{50} 7,300 nM, 31% activation), and at 1 μM was inactive at the β_2 AR. This highly selective β_3 AR agonist, L-757,793 has an EC_{50} value of 6.3 nM (70% activation) and binds to the β_3 AR with an IC_{50} value of 44 nM.

Table 2. Comparison of the β_3 AR Agonist Activity and β_1 and β_2 Binding Affinity for Sulfonamides **5k–u**



Compound	X	R^3	β_3 EC_{50}, nM (%act)[a]	β_1 Binding IC_{50}, nM[b]	β_2 Binding IC_{50}, nM[b]
5k	NH	Me	100 (63)	2,000	8,000
5l	NH	nPro	68 (33)	2,000	8,000
5m	NH	nHex	6.3 (70)	8,000	3,000
5n	NH	nOct	1.4 (60)	970	410
5o	NH	$(CH_2)_2Ph$	1.6 (55)	160	200
5p	O	nPro	150 (65)	60,000	10,000
5q	O	nHex	27 (70)	2,000	380
5r	O	$(CH_2)_2Ph$	67 (68)	7,000	820
5s	CH_2	nPro	67 (72)	60,000	9,000
5t	CH_2	nHex	18 (81)	8,000	10,000
5u	CH_2	$(CH_2)_2Ph$	18 (53)	10,000	2,000

[a]Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol. [b]Receptor binding assays were carried out with membranes prepared from CHO cells expressing the cloned human receptor in the presence of ^{125}I-iodocyanopindolol.

The urea L-757,793 **5m** was examined in a rising dose study in anesthetized rhesus monkeys.[17] L-757,793 was given by bolus injection at 15 minute intervals. L-757,793 elicited hyperglycerolemia (ED_{50} 0.2 mg/kg) with a maximum response equivalent to that of isoproterenol. No significant heart rate effects were seen up to the maximum dose tested (1 mg/kg).

When L-757,793 was administered orally (10 mg/kg) to dogs, no serum glycerol response was recorded. Plasma concentrations of L-757,793 were less than 10 nM. A large improvement was observed with the 4-iodophenyl compound **5j**. Its oral bioavailability in dogs (dosed 10 mg/kg po, 3 mg/kg iv) was 51%. This data supports our earlier findings, in the phenoxypropanolamine series,[10] that the urea moiety is detrimental to oral absorption.

In conclusion, we have shown that the 3-pyridylethanolamine L-757,793 is a potent β_3 AR agonist (EC_{50} 6.3 nM) with 1,300- and 500-fold selectivity over binding to the β_1 and β_2 ARs, respectively. Oral bioavailability of L-757,793 was poor; however, the impressive oral bioavailability (51%) of the 4-iodobenzenesulfonamide suggests that modification of the substituents on the benzenesulfonamide moiety has the potential to produce a compound with the desirable biological profile of L-757,793, and the pharmacokinetic properties necessary for an oral therapeutic agent. Work in this area is ongoing and will be published in due course.

Acknowledgment: We thank Mr. Paul Cunningham and Mr. Donald Hora, Jr. for expert technical assistance with the in vivo experiments, Ms. Amy Bernick for mass spectral analyses, Dr. Gerard Kieczykowski and Mr. Joseph Leone for preparing large quantities of key intermediates, and Professor James Grannemann (Wayne State University) for supplying the cloned human β_3 adrenergic receptor.

References and Notes

1. Present address: Schering Plough Research Institute, Kenilworth, NJ 07033, U.S.A.

2. For recent reviews see: Lowell, B. B.; Flier, J. S. *Annu. Rev. Med.* **1997**, *48*, 307; Strosberg, A. D.; Pietri-Rouxel, F. *Trends Pharmacol. Sci.* **1996**, *206*, 373; Arch, J. R. S.; Wilson, S. *Int. J. Obesity* **1996**, *20*, 191.

3. For reviews see: Dow, R. L. *Exp. Opin. Invest. Drugs* **1997**, *6*, 1811; Claus, T. H.; Bloom, J. D. *Ann. Rep. Med. Chem.* **1995**, *30*, 189.

4. Arch, J. R. S; Ainsworth, A. T.; Cawthorne, M. A.; Piercy, V.; Sennitt, M. V.; Thody, V. E.; Wilson, C.; Wilson, S. *Nature* **1984**, *309*, 163.

5. Liggett, S. B. *Mol. Pharmacol.* **1992**, *42*, 634.

6. See ref 9 for experimental details. Briefly, the human β_3 receptor was obtained from Professor J. Grannemann (Wayne State University). The human β_1 and β_2 receptors 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.; Lefkowsitz, 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_3 receptors) or 300–500 fmol/mg (β_1 and β_2 receptors). Agonist activity and binding affinity were assessed by measurement of cellular cAMP levels relative to isoproterenol and inhibition of ^{125}I-iodocyanopindolol binding, respectively.

7. 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.

8. 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.

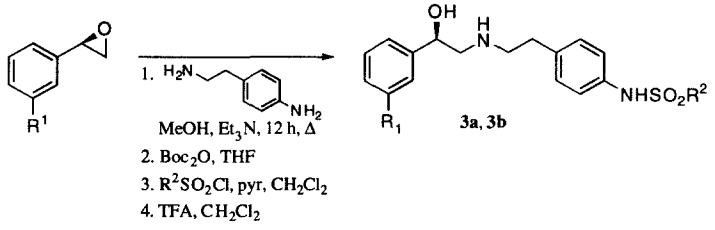
9. Fisher, M. H.; Amend, A. M.; Bach, T. J.; Barker, J. M.; Brady, E. J.; Candelore, M. R.; Carroll, D.; Cascieri, M. A.; Chiu, S.-H. L.; Deng, L.; Forrest, M. J.; Hegarty-Friscino, B.; Guan, X.-M.; Hom, G. J.; Hutchins, J. E.; Kelly, L. J.; Mathvink, R. J.; Metzger, J. M.; Miller, R. R.; Ok, H. O.; 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.

10. 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. J.; 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.

11. Presented in part at the 26th National Medicinal Chemistry Symposium, Richmond, VA, June 1998; Abstract D29.

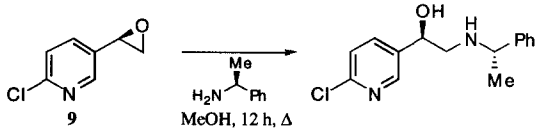
12. The phenethanolamines **3a** and 3-chloro analogs **3b** were synthesized from the commercially available chiral epoxides in a similar manner to that reported for the aryloxypropanolamines (see ref 7). Epoxide opening with *p*-aminophenethylamine was followed by selective Boc-protection of the resultant secondary amine. Treatment with the appropriate sulfonyl chloride and deprotection with TFA afforded the desired sulfonamides **3a** and **3b**.



13. The 3-pyridylethanolamines **5a–u** were prepared as optically active *R* enantiomers. Several pairs of *R* and *S* enantiomers in this 3-pyridylethanolamine series have been synthesized and their β_3 AR agonist activity examined. In each case, in line with expectation, the *R* isomer was 5–190 fold more potent than the respective *S* isomer. All final compounds were characterized by NMR, mass spectrometry and HPLC. For experimental details see: Fisher, M. H.; Naylor, E. M.; Weber, A. E. U.S. Patent 5 541 197, 1996.

14. Personal communication from Dr. Sander G. Mills.

15. The enantioselectivity of the chiral reduction was determined by opening the epoxide with *S*-(-)-α-methylbenzylamine. The diasteromeric excess of the resultant ethanolamine was calculated to be 90% from the ^1H NMR spectrum.



16. The 4-ureidobenzenesulfonyl chlorides were prepared either by treatment of the phenyl urea with chlorosulfonic acid or by addition of the amine to 4-(chlorosulfonyl)phenyl isocyanate. The 4-carbamoylbenzenesulfonyl chlorides were synthesized by addition of the alcohol to 4-(chlorosulfonyl)phenyl isocyanate. The 4-amidobenzenesulfonyl chlorides were prepared by treatment of the 4-amidobenzene with chlorosulfonic acid.

17. Male lean rhesus monkeys (n = 3) were fasted overnight and administered ketamine (10 mg/kg, im) for induction of anesthesia. Catheters were placed in the cephalic vein and femoral artery for intravenous drug administration and measurement of arterial pressure and heart rate. ECG leads were also attached for monitoring Lead II ECG and heart rate. While monitoring the cardiovascular parameters, Nembutal (20–25 mg/kg, iv) was given over 10 min for anesthesia. Heart rate was monitored for approximately 30 min until stable baseline values were obtained, at which time animals were administered a series of bolus doses of test compound in a vehicle consisting of 10% aqueous citric acid solution (10 mM), 30% PEG400, 60% saline. Bolus doses were separated by an interval of 15 min. Blood samples (2 mL) were collected from the femoral artery one min prior to the administration of compound and 14 min after each bolus dose. Serum glycerol was measured using an enzymatic colorimetric assay. After completion of the series of bolus doses of test compound, animals were administered an infusion of isoproterenol (100 μg/kg) over 15 min to elicit a maximal increase in serum glycerol.