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DISCOVERY OF L-755,507: A SUBNANOMOLAR HUMAN β_3 ADRENERGIC RECEPTOR AGONIST

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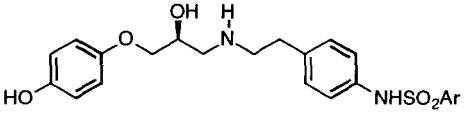
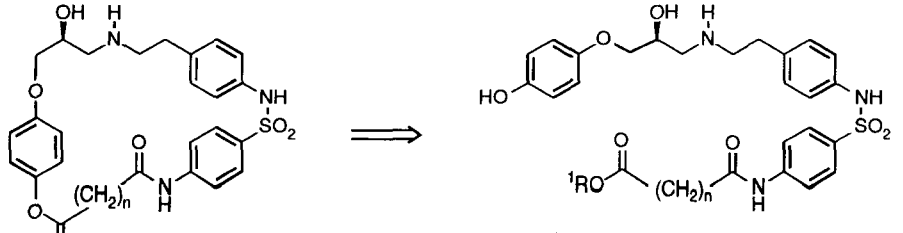
Abstract: A study of 4-acylaminobenzenesulfonamides in a cloned human β_3 adrenergic receptor assay resulted in the discovery of *n*-hexylurea, L-755,507 (**22**). This 0.43 nM β_3 agonist, which is > 440-fold selective over both β_1 and β_2 binding, is among the most potent human β_3 agonists reported to date.

In the preceding paper benzenesulfonamide derivatives **1** were reported as potent and selective agonists of the human β_3 adrenergic receptor.[2] During a study of conformationally constrained analogs of these compounds (**2**), the open chain esters **3** and acids **4** were prepared.[3,4] When tested in the human β adrenergic receptor assays,[5,6] esters **3** were highly potent β_3 agonists (β_3 EC_{50} 0.9–5.3 nM, 53–97% activation) that showed good selectivity over binding at the β_1 and β_2 receptors. The series of carboxylic acids **4** exhibited a slight reduction in potency (β_3 EC_{50} 5–15 nM), but intrinsic activity and selectivity were retained. This paper describes an extension of this work to include a variety of 4-acylaminobenzenesulfonamides, leading to the discovery of L-755,507 (**22**), which is among the most potent human β_3 adrenergic receptor agonists reported to date.



1 (Ar=Ph) β_3 EC_{50} 6.3 nM



2 n=1–6

3 (R^1=Me) β_3 EC_{50} 0.9–5.3 nM

4 (R^1=H) β_3 EC_{50} 5–15 nM

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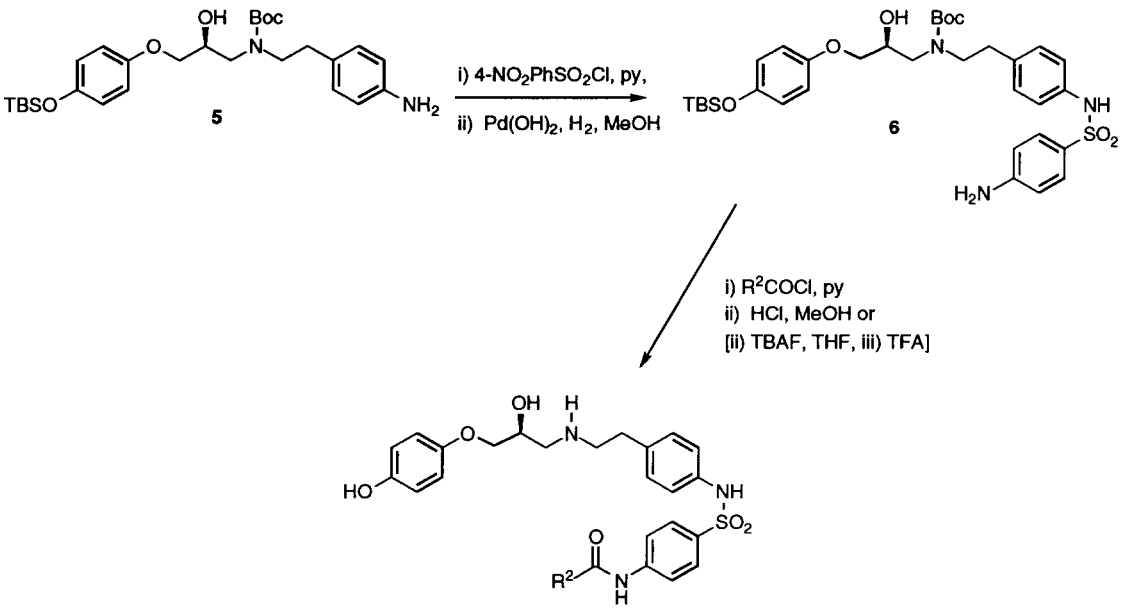
Scheme 1. Synthesis of amides **7–12** and carbamates **13–17**.

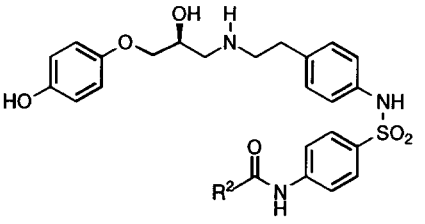


7–12 (R^2 = Alk, Ph)
13–17 (R^2 = AlkO)

Amides **7–12** and carbamates **13–17** were prepared from aniline **5**[2] by coupling with 4-nitrobenzenesulfonyl chloride, reduction to aniline **6**, selective acylation, and deprotection (Scheme 1). Removal of the silyl ether and *tert*-butylcarbamate protecting groups was effected either by treatment with methanolic hydrogen chloride or by sequential treatment with tetrabutylammonium fluoride solution and trifluoroacetic acid.[4,7]

As a reference, the parent aniline, prepared by deprotection of silyl ether **6**, was tested for activity at the cloned human β_3 adrenergic receptor and was found to be a moderately potent β_3 agonist (β_3 EC_{50} 17 nM, 100% activation). Acylation of the aniline, however, results in a significant increase in potency. This is demonstrated with a series of amides **7–12** and carbamates **13–17** and the in vitro data are summarized in Table 1. With the exception of acetamide **7** (β_3 EC_{50} 5 nM, 55% activation), all the amides were full agonists of the β_3 adrenergic receptor and were highly potent compounds (β_3 EC_{50} 1–7 nM). Isopropylamide **10** (β_3 EC_{50} 1.2 nM) was the most selective, exhibiting 600-fold and 375-fold selectivity over β_1 and β_2 binding, respectively.

Table 1. Activity of amides **7–12** and carbamates **13–17** at the cloned human β adrenergic receptors.



7-17

Compound	R^2	nM β_3 EC_{50} (% act)[a]	β_1 binding IC_{50}[b] (nM)	β_2 binding IC_{50}[b] (nM)
7	Me	5 (55)	1000	2000
8	Et	1.4 (100)	880	220
9	Pr	6.8 (100)	530	230
10	iPr	1.2 (100)	730	450
11	Hex	1.4 (90)	340	230
12	Ph	2.3 (98)	220	110
13	MeO	0.7 (100)	250	430
14	EtO	1.1 (99)	220	400
15	BnO	1 (100)	190	130
16	iPrO	2.6 (100)	240	210
17	HexO	11 (44)	190	110

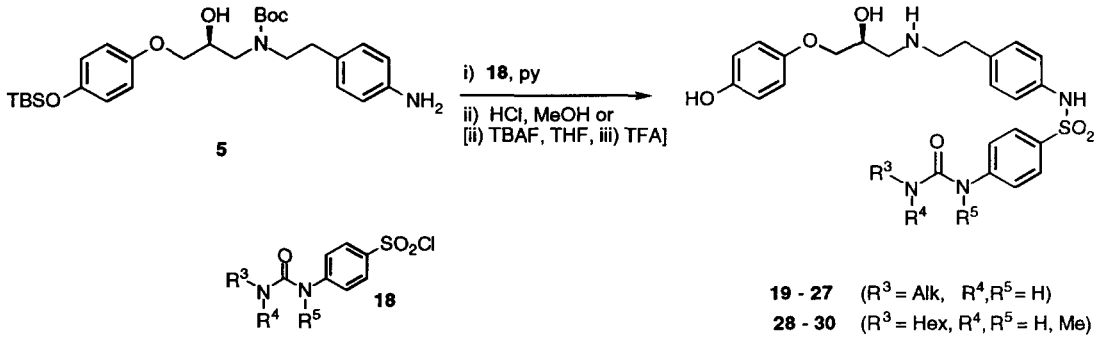
[a]Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol.
[b]Receptor binding assays were carried out with membranes prepared from CHO cells expressing the cloned human receptor in the presence of ^{125}I-iodocyanopindolol.

Similarly, carbamates **13–16** were full agonists of the β_3 adrenergic receptor with excellent potency (β_3 EC_{50} 1–3 nM). Methylcarbamate **13** was the most potent and selective compound in this series (β_3 EC_{50} 0.7 nM, 350-fold and 610-fold selective over β_1 and β_2 binding, respectively). None of the compounds shown in Table 1 exhibited any agonist activity at the β_2 receptor; however, with the exception of acetamide **7**, they were partial agonists of the β_1 receptor (45–68% activation; data not shown) and hence the series were not pursued further.

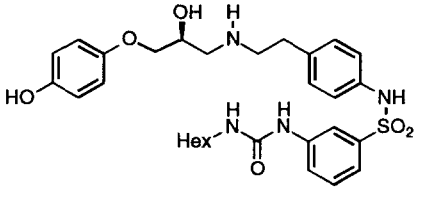
A series of alkylureas **19–30** were then prepared by reaction of aniline **5** directly with the preformed sulfonyl chloride **18**,[8] followed by deprotection as before (Scheme 2). These compounds showed interesting biological activity as shown in Table 2. Ureas **19–27** were highly potent agonists of the human β_3 adrenergic receptor (β_3 EC_{50} 0.43–5.2 nM) and in most cases were >100-fold selective over β_1 and β_2 binding. In our cloned assay the ureas were partial agonists at the β_3 receptor (49–67% activation). This apparent loss of intrinsic activity was not deemed significant, however, as efficacy varies with expression levels,[9] which are low in our assay,[5] and thus may underestimate lipolytic effects in vivo. Primary ureas **19–25** exhibited little agonist activity at the β_1 receptor (14–36% activation; data not shown), although ureas **26** and **27**, containing a secondary alkyl

substituent, did activate the β_1 receptor to a greater extent (76–83% activation). As with the amides and carbamates, there was no agonist activity at the β_2 receptor.

Scheme 2. Synthesis of ureas **19–30.**

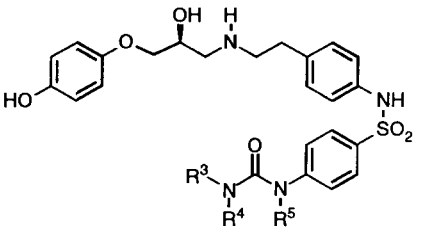


Notably, the *n*-hexyl urea **22**, L-755,507, displays an excellent activity profile as an extremely potent human β_3 adrenergic receptor agonist (β_3 EC_{50} 0.43 nM), with >440-fold selectivity over β_1 and β_2 binding. Furthermore, it is only a weak partial agonist at the β_1 receptor (β_1 EC_{50} 580 nM, 25% activation) with >1300-fold selectivity for β_3 agonist activity over β_1 agonist activity. L-755,507 also exhibits potent binding at the human β_3 receptor (β_3 IC_{50} 13 nM). In order to explore the SAR further, simple methylation of the urea moiety of L-755,507 was effected to give compounds **28-30**. These alkylated ureas showed enhanced intrinsic activity compared to the parent when tested in the β_3 adrenergic receptor assay (70–87% activation). N-Methylation of the terminal nitrogen resulted in a slight loss in potency (**28** β_3 EC_{50} 2.1 nM), while removal of the anilino hydrogen atom (**29** and **30**) led to a significant 15-fold and 23-fold loss in potency (β_3 EC_{50} 6.6–10 nM). The analogous hexyl carbamate (**17**, Table1) was also much less potent than the urea (**17** β_3 EC_{50} 11 nM). The 3-substituted derivative **31**, prepared as described above, not only exhibited a 10-fold loss in potency at the β_3 receptor relative to L-755,507 (**31** β_3 EC_{50} 4.6 nM, 46% activation), but also showed greatly reduced selectivity over binding at the β_1 and β_2 receptors (21-fold and 11-fold selective, respectively).



31

Table 2. Activity of ureas **19–30** at the cloned human β adrenergic receptors.



Compound	R^3	R^4	R^5	nM β_3 EC_{50} (% act)[a]	β_1 binding IC_{50}[b] (nM)	β_2 binding IC_{50}[b] (nM)
19	Me	H	H	1.4 (67)	350	400
20	Pr	H	H	1.1 (58)	540	540
21	nPent	H	H	5.2 (58)	300	350
22	**nHex**	**H**	**H**	**0.43 (52)**	**200**	**190**
23	nHept	H	H	2.8 (50)	200	200
24	Oct	H	H	1 (58)	150	170
25	MeOPr	H	H	2.4 (60)	1000	1000
26	iPr	H	H	1.2 (66)	150	700
27	cHex	H	H	1.9 (49)	260	5000
28	nHex	Me	H	2.1 (70)	800	720
29	nHex	H	Me	6.6 (87)	740	290
30	nHex	Me	Me	10 (71)	510	120

[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.

In summary, this paper describes the study of 4-acylaminobenzenesulfonamides as human β_3 adrenergic receptor agonists. The study culminated in the discovery of L-755,507, which was a highly potent subnanomolar human β_3 adrenergic receptor agonist. The compound also showed excellent selectivity for the β_3 receptor over the β_1 and β_2 receptors. Based on these data, L-755,507 was selected for further in vitro and in vivo evaluation in rhesus monkeys in order to study the effect of human β_3 adrenergic receptor agonists on lipolysis, metabolic rate, and energy expenditure in primates. The results of this study will the topic of a future publication.[10]

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References and Notes:

1. Present address: Schering Plough Research Institute, 2015 Galloping Hill Rd, Kenilworth, NJ 07033.

2. 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.* preceding paper.

3. Parmee, E. R.; Ok, H. O.; Szumiloski, J.; Candelore, M. R.; Tota, L.; Deng, L.; Strader, C. D.; Baum, M. W.; Doss, G. A.; Wyvratt, M. J.; Fisher, M. H.; Weber, A. E. *Abstracts of Papers*, 213th National Meeting of the Amercan Chemical Society, CA; American Chemical Society: San Francisco, CA, April 1997; Abstract MEDI 031.

4. All compounds were characterized by ^{1}H NMR, mass spectrometry, and HPLC analysis prior to submission for biological evaluation.

5. The human β_3 receptor was obtained from Professor J. Grannemann (Wayne State Universtiy), Granneman, J. G.; Lahners, K. N.; Rao, D. D. *Mol. Pharmacol.* **1992**, *42*, 964-970. 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*, 7920-7924 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*, 46-50. 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-cyanopindolol binding, respectively.

6. Compounds were screened for their ability to stimulate increases in cAMP in CHO cells expressing the cloned human β_3 adrenergic receptor, but not in cells expressing the cloned human β_1 or β_2 adrenergic receptor.

7. 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.

8. 4-Ureidobenzenesulfonyl chlorides **18** were prepared either by treatment of the phenyl urea with chlorosulfonic acid, or by addition of an amine to 4-(chlorosulfonyl)phenyl isocyanate.

9. Wilson, S.; Chambers, J. K.; Park, J. E.; Ladurner, A.; Cronk, D. W.; Chapman, C. G.; Kallender, H.; Browne, M. J.; Murphy, G. J.; Young, P. W. *J. Pharm. Exper. Ther.* **1996**, *279*, 214–221.

10. For discussion of these results see; 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.* manuscript submitted for publication.