Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2111–2116

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

3-PYRIDYLOXYPROPANOLAMINE AGONISTS OF THE β_3 ADRENERGIC RECEPTOR WITH IMPROVED PHARMACOKINETIC PROPERTIES

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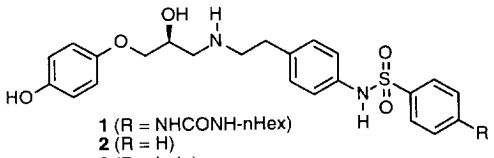
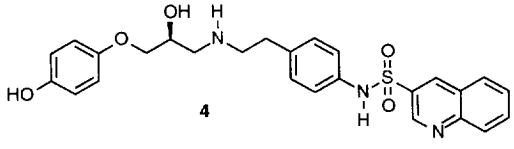
Received 28 May 1998; accepted 1 July 1998

Abstract: Pyridyloxypropanolamines L-749,372 (**8**, β_3 EC_{50} = 3.6 nM) and L-750,355 (**29**, β_3 EC_{50} = 13 nM) are selective partial agonists of the human receptor, with 33% and 49% activation, respectively. Both stimulate lipolysis in rhesus monkeys (ED_{50} = 2 and 0.8 mg/kg, respectively), with minimal effects on heart rate. Oral bioavailability in dogs, 41% for L-749,372 and 47% for L-750,355, is improved relative to phenol analogs.

Increasing metabolic rate through activation of β_3 adrenergic receptors (β_3 ARs) on the surface of adipocytes is an attractive approach to the treatment of obesity.[2] Recently, we disclosed a series of potent and selective agonists of the human β_3 AR, including benzenesulfonamides **1–4**.[3–5] The hexylureido derivative L-755,507 (**1**) is a potent partial agonist of the human β_3 AR (EC_{50} = 0.43 nM; 52% of the maximum response elicited by isoproterenol, a nonselective full agonist), with >400-fold selectivity for activation of the β_3 AR versus activation of or binding to β_1 and β_2 ARs.[4] In addition, intravenous administration of L-755,507 to rhesus monkeys was shown to elicit lipolysis and elevation of metabolic rate.[5] Herein we describe the pharmacokinetic profiles of these phenolic β_3 AR agonists and our efforts to identify derivatives with improved metabolic stability.[6]



1 (R = NHCONH-nHex)
2 (R = H)
3 (R = Iodo)



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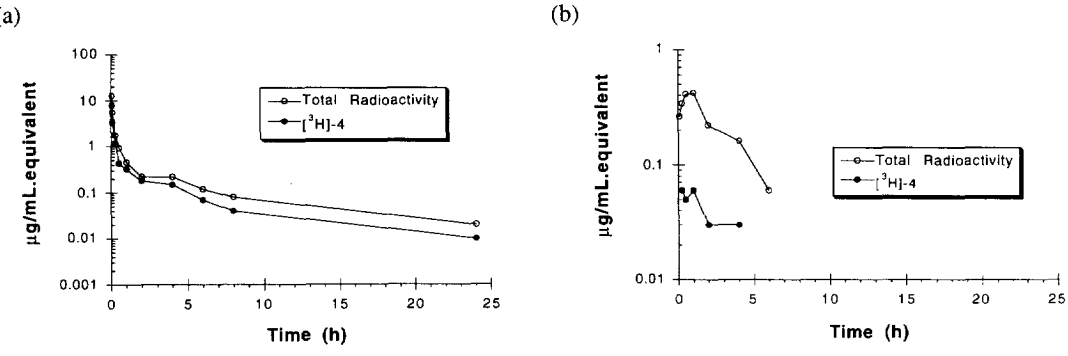
Initially, the pharmacokinetic profile of the unsubstituted benzenesulfonamide **2** was determined in rats. Following oral administration (1 mg/kg), plasma concentrations of agonist **2** were below the limit of detection (10 ng/mL). This derivative exhibited high plasma clearance (>200 mL/min/kg) and a half-life of 25 min, following intravenous administration in rats (1 mg/kg). Incubation of **2** (20 μM) with rat liver microsomes (1 mg/mL) and uridine diphosphate glucuronic acid (3 mM) resulted in rapid glucuronidation, with only 10% of the unchanged compound remaining after 30 min. Surprisingly, the in vitro rate of glucuronidation appeared to be dependent on the structure of the sulfonamide moiety as the iodo derivative **3** and 3-quinolinyl derivative **4** were much more stable than **2** toward glucuronidation (>70% unchanged) when incubated under similar conditions.

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00381-3

L-755,507 (**1**) and quinoline derivative **4**, a β_3 AR agonist (EC_{50} = 1.3 nM, 73% activation) with ≥ 180-fold selectivity for activation of the β_3 AR over activation of or binding to β_1 and β_2 ARs,[3] were chosen for further pharmacokinetic evaluation, and radiolabeled analogs [^{3}H]-**1** and [^{3}H]-**4** were prepared.[7] Following oral administration to bile duct cannulated rats, absorption of radioactivity was 45% (44% and 1% recovered in bile and urine, respectively) for [^{3}H]-**4**, but only 1.5% for [^{3}H]-**1**, suggesting that the polar and presumably highly solvated urea moiety was detrimental to absorption. Poor absorption was also observed for urea **1** in dogs, where its oral bioavailability was estimated to be <1%.

Although quinoline **4** exhibited improved stability over phenyl derivative **2** with respect to hepatic glucuronidation, as shown in the in vitro studies, its oral bioavailability in rats remained low (3%) due to a significant first pass effect following oral dosing in this species. As illustrated in Figure 1, when [^{3}H]-**4** was administered intravenously to rats (5 mg/kg), plasma concentrations of total radioactivity and radioactivity due to [^{3}H]-**4** were similar. In contrast, following oral administration to rats (10 mg/kg), concentrations of total radioactivity were nearly 10-fold higher than radioactivity due to intact [^{3}H]-**4**, suggesting that a large fraction of the radioactivity was due to the presence of metabolites. This observation was confirmed in a separate experiment in which portal vein cannulated rats were dosed intraduodenally with [^{3}H]-**4** and radioactivity profiles in portal blood were examined. The major metabolite, which accounted for ~40% of the administered radioactivity, could be converted to [^{3}H]-**4** by treatment with β-glucuronidase. These results suggest that glucuronidation remained problematic, most likely due to the first pass effect in intestines. The oral bioavailability of **4** in dogs was slightly higher (13%), but not satisfactory. Therefore, the identification of derivatives which lacked the phenolic moiety became a high priority.

Figure 1. Plasma concentrations of total radioactivity (open circles) and [^{3}H]-**4** (filled circles) following 5 mg/kg intravenous (a) and 10 mg/kg oral (b) administration in rats



A wide variety of monosubstituted phenoxypropanolamine analogs were explored. Derivatives **5–29** were synthesized in a manner analogous to that used for the preparation of phenol derivatives **1–4**.[3,4,8] Final products were characterized by NMR, mass spectrometry, and HPLC. All compounds were screened for their ability to stimulate increases in cAMP in Chinese hamster ovary (CHO) cells expressing the cloned human β_3 receptor.[9] The activity of an agonist at the β_3 AR is better 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 assay accurately predicts the lipolytic potential of compounds in native adipocytes.[5] Because these

derivatives generally have very low efficacy at β_1 and β_2, binding affinities to these ARs were routinely measured, with efficacy at the β_1 and β_2 ARs determined for selected compounds.[10]

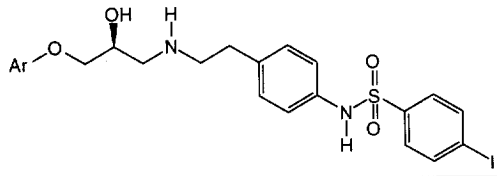
Derivatives in which the phenol was replaced with other hydrogen bonding groups such as $-NH_2$, -COOH, $-CH_2OH$, $-CONH_2$, $-SO_2NH_2$, AcNH-, and $MeSO_2NH$- were inactive toward activation of the β_3 AR at 1 μM. As illustrated in Table 1 for the 4-iodobenzenesulfonamide series, unsubstituted analog **5** and methoxy analog **6** also had very low efficacy at the β_3 AR. The corresponding 4-fluoro derivative **7** is an 18 nM partial agonist with 41% activation; however, this compound is only fivefold selective over binding to the β_2 AR. Modification of the sulfonamide moiety in this series did not lead to a substantial improvement in selectivity.

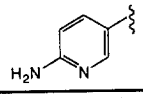
Heterocyclic derivatives were also extensively evaluated, leading to the identification of two potent analogs, pyridine derivative **8** and aminopyridine derivative **9** (Table 1). The former, L-749,372, is a partial agonist (EC_{50} = 3.6 nM, 33% activation) with 270- and 30-fold selectivity for activation of the β_3 AR over binding to β_1 and β_2 ARs, respectively. This derivative binds to the human β_3 AR with an IC_{50} of 34 nM. It shows no agonist activity at the β_2 AR at 1 μM, but is a partial agonist at the β_1 AR, with an EC_{50} of 430 nM (33% activation). Aminopyridine **9** (β_3 EC_{50} = 7.8 nM, 83% activation) is more efficacious at the β_3 AR than L-749,372, with 130-fold selectivity over binding to the β_1 AR, but only threefold selectivity over binding to β_2.

These results suggest that both steric factors (e.g., methoxy **6** vs. fluoro **7**) and hydrogen bond accepting ability may be important for β_3 agonist activity in the aryloxypropanolamine series. Because β_2 binding affinity appears to be relatively insensitive to changes in this portion of the molecule while β_3 affinity is somewhat decreased in compounds with agonist activity (e.g., **7–9**), selectivity for β_3 over β_2 generally decreased.

In order to further optimize these heterocyclic derivatives, a number of sulfonamide analogs were prepared

Table 1. Activity of aryloxypropanolamine analogs of 4-iodobenzenesulfonamide **3**

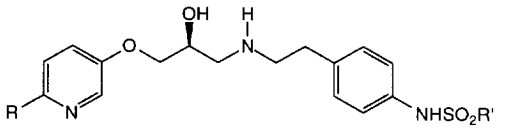


Compound	Ar	β_3 EC_{50} (%act)[a] (nM)	β_1 Binding IC_{50}[b] (nM)	β_2 Binding IC_{50}[b] (nM)
3	4-OH-Ph	1.6 (73)	170	120
5	Ph	120 (10)	80	50
6	4-OMe-Ph	(5)[c]	860	150
7	4-F-Ph	18 (41)	910	100
8		3.6 (33)	1000	110
9		7.8 (83)	1000	28

[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. [c]Single point data, % activation at 100 nM.

in both the pyridine and aminopyridine series. As shown in Table 2, in both series, the unsubstituted benzenesulfonamides (**10** and **20**) and the quinolinesulfonamides (**11** and **21**) were less potent than the iodo leads. In contrast, urea analogs, **12** and **22**, were potent and selective partial agonists, but these derivatives were not pursued further due to the poor absorption inherent in the urea series. In the pyridine series, none of the other analogs tested, including halogen derivatives **13–16**, methoxy derivative **17**, and alkyl derivatives **18** and **19**, showed significant improvements in both potency and efficacy.

Table 2. Activity of pyridine and aminopyridine derivatives



Compound	R	R'	β_3 EC_{50} (%act)[a] (nM)	β_1 Binding IC_{50}[b] (nM)	β_2 Binding IC_{50}[b] (nM)
10	H	Ph	32 (34)	1300	750
11	H	3-Quinolinyl	32 (35)	1300	490
12	H	4-NHCONH-nHex-Ph	3.8 (26)	600	430
13	H	4-F-Ph	(18)[c]	1000	900
14	H	4-Cl-Ph	73 (44)	1000	430
15	H	4-Br-Ph	22 (32)	2000	230
16	H	3,4-dichloro-Ph	(24)[c]	290	360
17	H	4-OMe-Ph	7.0 (16)	1000	520
18	H	4-Et-Ph	(23)[c]	590	94
19	H	4-isoPr-Ph	85 (39)	1000	410
20	NH_2	Ph	400 (50)	10,000	630
21	NH_2	3-Quinolinyl	43 (50)	6300	1300
22	NH_2	4-NHCONH-nHex-Ph	6.0 (42)	900	640
23	NH_2	4-F-Ph	(26)[c]	2000	1000
24	NH_2	4-Cl-Ph	(30)[c]	2000	990
25	NH_2	4-Br-Ph	13 (59)	5300	380
26	NH_2	3,4-dichloro-Ph	43 (38)	470	380
27	NH_2	4-OMe-Ph	22 (37)	5500	620
28	NH_2	4-Et-Ph	(26)[c]	440	110
29	NH_2	4-isoPr-Ph	13 (49)	1800	540

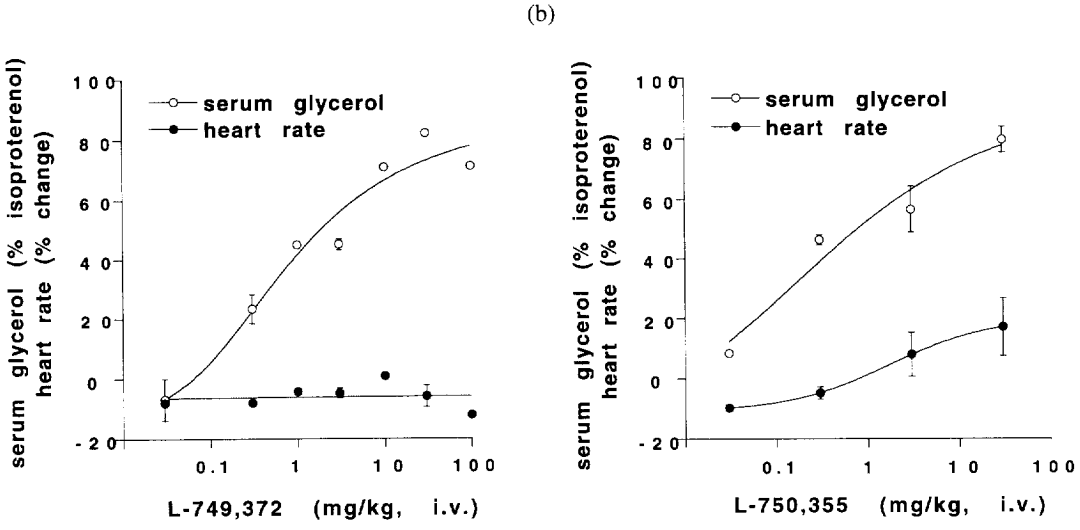
[a]Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol. [b]Receptor binding assays were carried out with membranes prepared from CHO cells expressing the cloned human receptor in the presence of ^{125}I-iodocyanopindolol. [c]Single point data, % activation at 100 nM.

In the aminopyridine series, improving selectivity over β_2 AR binding while maintaining potency at β_3 was a primary objective. Iodo lead **9** proved to be optimal with regard to activity at the β_3 AR, and analogs typically showed reductions in both potency and efficacy. For example, fluoro, chloro, 3,4-dichloro, and ethyl derivatives **23**, **24**, **26**, and **28** all showed decreased potency at the β_3 AR; however, bromo analog **25** was

only twofold less potent (59% activation) and showed improved (30-fold) selectivity over the β_2 AR. The 4-methoxy derivative **27** had similar selectivity, but reduced potency and efficacy at the β_3 AR. Isopropyl analog **29** showed good potency at the β_3 AR (EC_{50} = 13 nM, 49% activation), and was the most selective (40-fold) derivative in this series. This derivative, L-750,355, is a partial agonist at the β_1 AR (EC_{50} = 3200 nM, 37% activation) and shows no activation of the β_2 AR at 1 μM.

Although L-749,372 (**8**) and L-750,355 (**29**) are only partial agonists in the β_3 AR functional assay (33% and 49% activation, respectively), they both showed nearly full lipolytic responses when administered intravenously to rhesus monkeys (Figure 2).[11] ED_{50}'s for glycerolemia were 2 and 0.8 mg/kg, respectively, with a maximum response 80% that of the full agonist isoproterenol. Heart rate effects were minimal, with no changes noted for L-749,372 and a 17% increase for L-750,355 at the highest dose tested (30 mg/kg).

Figure 2. Effects of L-749,372 (a) and L-750,355 (b) on plasma glycerol and heart rate in anesthetized rhesus monkeys following intravenous administration



While the oral bioavailability in rats (3 mg/kg i.v., 10 mg/kg p.o.) of L-750,355 was not improved relative to quinoline **4** (4% vs. 3%), that of L-749,372 was slightly better (13%). Significant improvements in oral bioavailability were observed in dogs (3 mg/kg i.v., 10 mg/kg p.o.). For the two 3-pyridyl derivatives, oral bioavailabilities were three- to fourfold higher than that of quinoline **4**: 41% for L-749,372 and 47% for L-750,355. Thus replacement of the phenol in the phenoxypropanolamine series with the pyridine moiety does result in an increase in oral bioavailability, in particular, in dogs.

In conclusion, we have identified two pyridyloxypropanolamine partial agonists of the human β_3 AR, L-749,372 and L-750,355. Both compounds stimulate lipolysis in the rhesus monkey with minimal effects on heart rate. In addition, both showed improved pharmacokinetic profiles in the dog.

Acknowledgment: We thank Dr. William Feeney, Mr. Paul Cunningham, and Mr. Donald Hora, Jr. for assistance with the in vivo experiments, Ms. Amy Bernick for mass spectral analyses, and Professor James G. Grannemann (Wayne State University) for supplying the cloned human β_3 adrenergic receptor.

References and Notes

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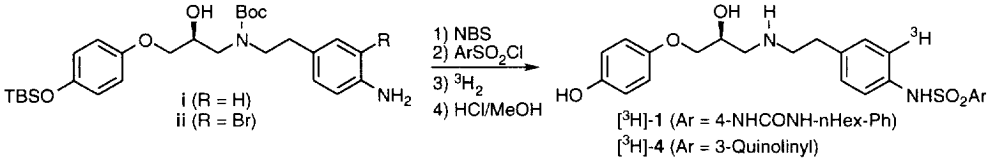
6. Presented in part at the 213th National Meeting of the American Chemical Society, San Francisco, CA, April 1997; Abstract MEDI 0168.

7. Synthesized from aniline **i** (Ref. 3) by treatment with *N*-bromosuccinimide in benzene to give **ii**, followed by acylation with the appropriate sulfonyl chloride, treatment with tritium gas over palladium on calcium carbonate, and deprotection with methanolic hydrogen chloride:



8. 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.

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10. 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, repectively.

11. See Ref. 5 for experimental details. Due to the small signal to noise ratio in conscious animals, this assay is run in anesthetized rhesus, which effectively precludes oral dosing. Briefly, male lean rhesus monkeys (n = 3) were fasted for 24 h and anesthetized. An intraveneous catheter was placed in a saphenous vein for the administration of test compounds and ECG leads were connected for the continuous measurement of heart rate. Heart rate was monitored for approximately 30 minutes until stable baseline values were obtained, at which time animals were administered a series of rising dose infusions (0.1 mL/min) of test compound over a 15-min period. Infusion periods were separated by an interval of approximately 20 sec. Blood samples (2 mL) were collected from the femoral artery 1 min prior to the initiation of infusions and 14 min into each infusion period. Serum glycerol was measured using an enzymatic colorimetric assay.