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L-770,644: A POTENT AND SELECTIVE HUMAN β_3 ADRENERGIC RECEPTOR AGONIST WITH IMPROVED ORAL BIOAVAILABILITY

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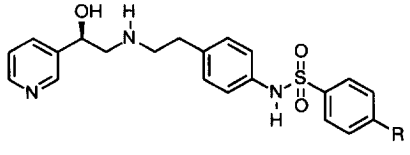
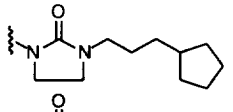
Abstract: L-770,644 (**9c**) is a potent and selective agonist of the human β_3 adrenergic receptor (EC_{50} = 13 nM). It shows good oral bioavailability in both dogs and rats (%F = 27), and is a full agonist for glycerolemia in the rhesus monkey (ED_{50} = 0.21 mg/kg). Based on its desirable in vitro and in vivo properties, L-770,644 was chosen for further preclinical evaluation.

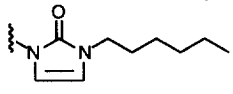
Stimulation of β_3 adrenergic receptors (β_3 ARs) on the surface of adipocytes evokes lipolysis and activation and upregulation of the uncoupling protein UCP1, which leads to a net increase in energy utilization. Thus, β_3 AR agonists may prove useful for the treatment of obesity.[2] Testing of this concept in the clinic has been hindered by the lack of β_3 AR agonists selective for the human receptor. Recently, we have disclosed potent and selective human β_3 AR agonists, but these compounds typically suffer from poor pharmacokinetic properties.[3–7] For example, L-766,892 and L-764,646 are potent β_3 AR agonists (EC_{50} = 5.7 and 14 nM, respectively); however, oral bioavailabilities of both compounds in the dog is modest (%F = 17 and 12, respectively, following oral administration in 0.05 M citric acid/0.05M hydrochloric acid solution).[6,7] The low oral bioavailability is due in part to extensive metabolism. In order to further reduce oxidative metabolism of the imidazolidinone and imidazolone rings (addition of oxygen to a ring carbon), the corresponding tetrazolone derivatives were synthesized. Herein we report the synthesis and biological activity of the resultant tetrazolone β_3 AR agonists.



L-766,892: R =



L-764,646: R =



The synthesis of tetrazolones **9a-c** is outlined in Scheme 1.[8] Alkylation of phenyltetrazolone **2**, obtained from phenylisocyanate and aluminum azide,[9] with the appropriate iodoalkanes **3a–c** afforded the *N*-alkylated tetrazolones **4a–c**. For the straight chain derivatives, **4a** (R = *n*-hexyl) and **4b** (R = *n*-octyl), direct chlorosulfonylation with chlorosulfonic acid gave the requisite sulfonyl chlorides **7a,b**. This method failed when

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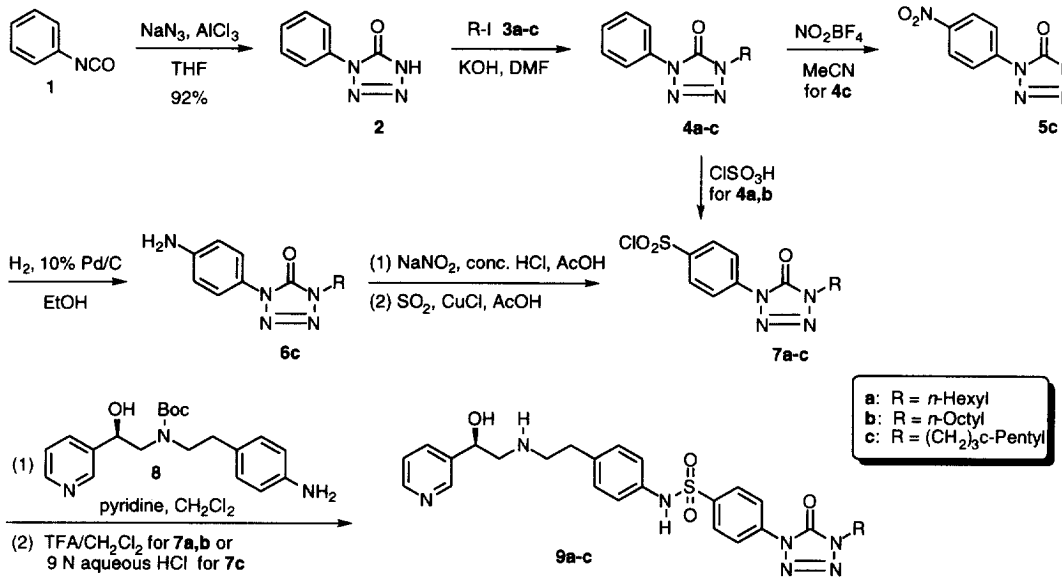
the sidechain was branched, as in tetrazolone **4c** (R = cyclopentylpropyl), which necessitated the development of a milder methodology for introducing the sulfonyl chloride functionality. Therefore, the phenyl ring was treated with nitrosonium hexafluoroborate to produce 4-nitrophenyltetrazolone **5c** as the major isomer. Palladium catalyzed hydrogenation of the nitro group gave the aniline in quantitative yield. Subsequent diazotization and reaction with sulfur dioxide-copper (I) chloride at -20 °C to room temperature[10] gave the requisite sulfonyl chloride. Standard sulfonamide formation with aniline **8**[5] followed by treatment with acid to remove the BOC protecting group gave the desired final compounds **9a–c**.

Scheme 1.



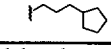
Based on SAR studies in the related imidazolidinone and imidazolone series,[6,7] the *n*-hexyl, *n*-octyl, and 3-cyclopentylpropyl tetrazolones were targeted for evaluation. Their activity at the cloned human β ARs is summarized in Table 1.[11] The activity of an agonist at the β_3 AR is better described by its ability to stimulate adenylyl cyclase in a functional assay (EC_{50}), 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.[3]

All three derivatives are partial agonists of the human β_3 AR (57–75% activation relative to the full agonist isoproterenol). They are much less efficacious at both the human β_1 and β_2 ARs. As was previously observed with other series,[6] the *n*-octyl analog **9b** was more potent than the n-hexyl analog **9a**. Cyclopentylpropyl derivative **9c** is only twofold less potent than **9b**; however, it is more selective, in particular, over partial agonist activity at the β_1 AR and binding to the β_2 AR. The activity and selectivity of the tetrazolone derivatives is comparable to that of the corresponding imidazolidinones and imidazolones, suggesting that these compounds bind to the receptor in a similar fashion.

The pharmacokinetic properties of tetrazolones **9b** and **9c** were determined in dogs.[12] Oral

bioavailabilities were 41 and 27%, respectively, which represented a significant improvement relative to L-766,892 and L-764,646. In addition, the half-life of the former compound was 3.5 h and of the latter, 3.6 h. Both compounds evoked hyperglycerolemia when administered orally to dogs at 10 mg/kg.

Table 1. Activity of tetrazolones **9** at the cloned human β ARs.

		β3		β1		β2	
Cmpd	R	EC_{50} (%act)[a] (nM)	Binding IC_{50}[b] (nM)	EC_{50} (%act)[a] (nM)	Binding IC_{50}[b] (nM)	EC_{50} (%act)[a] (nM)	Binding IC_{50}[b] (nM)
9a	*n*-Hexyl	30 (67)	n.d.[c]	(19)[d]	5000	(9)[d]	3300
9b	*n*-Octyl	6.3 (57)	n.d.[c]	370 (31)	1400	(15)[d]	570
9c		13 (75)	92	1900 (33)	3400	1800 (26)	1600

[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]Not determined.
[d]Single point data, % activation at 10 μM.

Because tetrazolone **9c** is more selective than **9b**, this derivative, L-770,644, was chosen for further study. L-770,644 binds to the β_3 AR with an IC_{50} of 92 nM, and is >100-fold selective for β_3 AR agonist activity when tested against a panel of receptors and ion channels, with the exception of α1a and α1b (44- and 56-fold, respectively) and dopamine D2 and D3 (34- and 15-fold, respectively). Its oral bioavailability in the rat was found to be 27%. In addition, intravenous administration to rhesus monkeys[13] evokes hyperglycerolemia (ED_{50} = 0.21 mg/kg), with a maximum response equivalent to that of the full agonist isoproterenol. Heart rate effects are minimal. A significant increase of 16% was seen at the highest dose tested (30 mg/kg).

In conclusion, L-770,644 is a potent and selective β_3 AR agonist, both in vitro and in vivo, which has good oral bioavailability in two species. Based on these data, L-770,644 was selected for extensive preclinical evaluation.

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12. Compounds were dosed orally at 10 mg/kg as a solution in 0.1 M aqueous citric acid, and intraveneously at 3 mg/kg in a vehicle consisting of polyethylene glycol 400 (PEG400), ethanol and normal saline. Blood samples were collected prior to dosing and at 5 (iv only), 15 and 30 min and 1, 2, 6, 8, and 24 h post dosing, for determination of plasma glycerol concentrations using a Sigma Triglyceride (GPO-TRINDER) assay kit and plasma drug concentrations by LC/MS/MS.

13. Due to the small signal to noise ratio in conscious animals, this assay is run in anesthetized rhesus monkeys, which effectively precludes oral dosing. Male lean rhesus monkeys (n = 2–4 per group) 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 min 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. Under these conditions, for isoproterenol the ED_{50} for hyperglycerolemia is 3 μg/kg and the ED_{50} for tachycardia is 0.2 μg/kg.[3]