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Design of a novel pyrrolidine scaffold utilized in the discovery of potent and selective human β_3 adrenergic receptor agonists

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ABSTRACT

A novel class of human β_3-adrenergic receptor agonists was designed in effort to improve selectivity and metabolic stability versus previous disclosed β_3-AR agonists. As observed, many of the β_3-AR agonists seem to need the acyclic ethanolamine core for agonist activity. We have synthesized derivatives that constrained this moiety by introduction of a pyrrolidine. This unique modification maintains human β_3 functional potency with improved selectivity versus ancillary targets and also eliminates the possibility of the same oxidative metabolites formed from cleavage of the N–C bond of the ethanolamine. Compound **39** exhibited excellent functional β_3 agonist potency across species with good pharmacokinetic properties in rat, dog, and rhesus monkeys. Early de-risking of this novel pyrrolidine core (**44**) via full AMES study supports further research into various new β_3-AR agonists containing the pyrrolidine moiety.

The β_3-adrenergic receptor (β_3-AR), also referred to as ADRB3, is present mainly in adipose tissue and is involved in the regulation of lipolysis and thermogenesis.[1,2] Consequently, β_3-AR agonists were extensively explored in the 1980's and 1990's for treatment of obesity and type 2 diabetes.[3] However, recent studies have shown that β_3-AR's are not only expressed in adipocytes but also in the bladder detrusor muscle and the lining of the gastrointestinal tract.[4,5] As a result, new therapeutic applications of β_3-AR agonists for the treatment of overactive bladder (OAB) and irritable bowel syndrome (IBS) have recently been explored.[6,7] Multiple companies have reported β_3-AR agonists over the past few decades, which has led to innovative ideas in the attempt to discover structurally unique β_3-AR agonists in this crowded competitive arena (Fig. 1).[8]

We present a recent discovery of a new pyrrolidine variation of the secondary amine moiety that appears as a conserved core of many reported β_3-AR agonists (Fig. 2). This modification affords analogs that maintain good potency and selectivity at the human β_3-AR while unlocking new prospects for research that could lead to a drug candidate.

In the late 1990's, Merck reported a series of potent and selective sulfonamide derived human β_3-AR agonists for the possible treatment for obesity.[13] Clinical candidate L-796,568 was discovered which possessed good functional human β_3-AR agonist potency and ~1000-fold selectivity over β_1 and β_2 adrenergic receptors. In preclinical species, however, L-796,568 exhibited

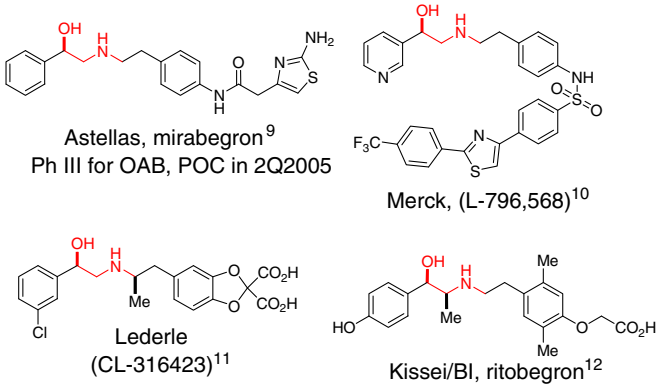

Figure 1. Examples of β_3-AR agonists (conserved acyclic ethanolamine pharmacophore in red). See above-mentioned references for further information.

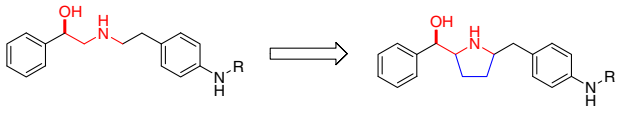

Figure 2. Pyrrolidine derived scaffold (blue highlights modification to acyclic ethanolamine).

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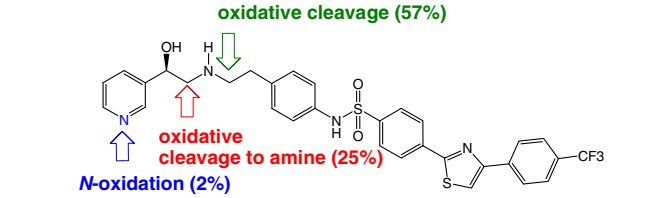
moderate oral bioavailabilities in rats and dogs and low oral bioavailability in rhesus.[14] To identify possible metabolic pathways which may be causing the high clearance and moderate oral bioavailability in rats, studies to determine oxidative metabolism in rat liver microsomes were performed.[15] These studies revealed that the majority of metabolites were derived from cleavage of the central C–N bond from the ethanolamine which ultimately led to identification of a carboxylic acid metabolite and a primary amine metabolite (Fig. 3). This oxidative metabolism may be a contributing factor to the high clearance and moderate oral bioavailability seen in rats. Moreover, the released metabolites may have untoward biological effects in animals. Blockade or modification of this metabolic pathway may therefore improve the pharmacokinetic profiles of acyclic β_3-AR agonists and prevent formation of metabolites such as those observed from L-796,568.

Previous reports have described the effects of simple substituents at either of the two carbons adjacent to the secondary amine,[11,12] and we sought to understand the effects of substituents on both sides of the amine. We therefore synthesized sulfonamide **4** (as a mixture of four diastereomers) which is a close analog of **3** (Fig. 4).[16] Unfortunately, the introduction of methyl substituents onto each of the carbons adjacent to the secondary amine resulted in a complete loss of β_3-AR activity which may be attributed to steric interactions between the two methyl substituents that induce a rotational conformation not favored by the β_3-AR.

If this hypothesis is correct, it is possible that restricting the rotation of the acyclic ethanolamine may result in a favorable conformation for β_3-AR agonist functional activity while



Pharmacokinetics

	Rat	Dog	Rhesus
Clp(ml/min/kg):	62	20	19
$t_{1/2}$(h):	8.1	14	9
Vd (L/kg):	23	12	6.2
PO AUC_N (μM•hr/mpk):	0.07	0.64	0.04
F (%)	17	27	4

Figure 3. Metabolism of L-796,568 observed in rats.[15]

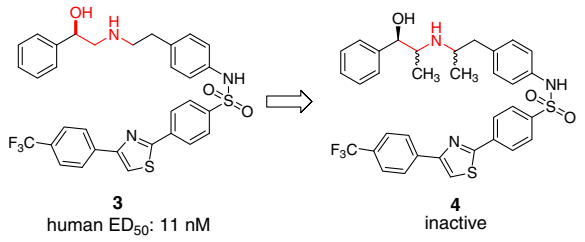


Figure 4. Di-methyl substituted analog **4**.

simultaneously modulating the metabolic pathways observed with L-796,568. Observation of molecular models suggested that a five-membered ring may best mimic the conformations of the acyclic ethanolamine. Consequently, chemistry was developed to synthesize a pyrrolidine core. Initially, the stereochemistry of the disubstituted pyrrolidine ring would need to be investigated to determine the preferred stereoisomer for β_3-AR agonist activity. A cross metathesis synthetic route using two substituted vinyl intermediates would afford the foundation from which all isomers could be obtained using a common synthetic sequence.

The synthesis of four distinct vinyl stereoisomer precursors began by treating commercially available 3-chlorobenzaldehyde (**5**) with vinyl magnesium bromide to afford vinyl alcohol **6** (Scheme 1). Protection of the hydroxyl group was accomplished with *tert*-butyl, dimethyl silyl chloride in the presence of triethylamine. Aldehyde **7** was obtained by oxidative cleavage of the vinyl group with ozone and subsequent reduction of the ozonide using triphenylphosphine. After formation of sulfinimide **8**[17] treatment with vinyl magnesium bromide afforded sulfinamide **9** as a mixture of all four diastereomers which were separated using silica gel column chromatography. Protecting group manipulation was then carried out on all four isomers to afford the *N*-Boc-protected compounds **10–13** which were then poised to undergo the desired cross metathesis reaction.

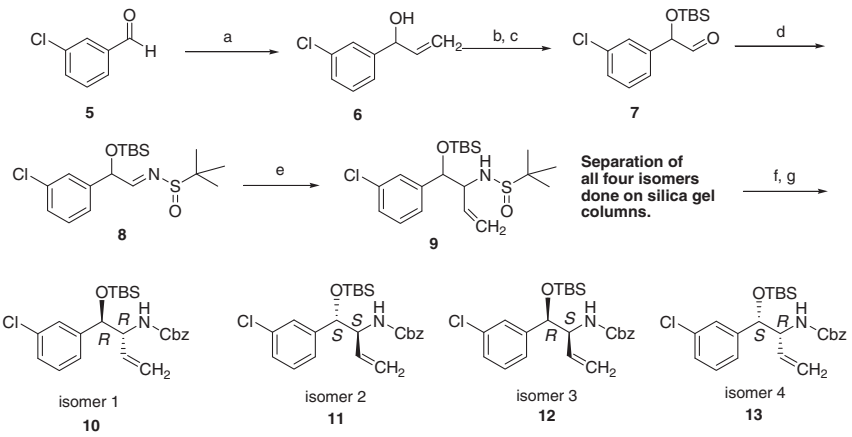
Preparation of the second vinyl intermediate is shown in Scheme 2. Intermediate **15** was obtained upon treatment of commercially available methyl (4-aminophenyl) acetate (**14**) with benzyl chloroformate followed by ester saponification with 5.0 N lithium hydroxide. The carboxylic acid was then converted to the Weinreb amide[18] (**16**) using standard EDC coupling with *N*,*O*-methylhydroxylamine. After purification via silica gel flash column chromatography, the Weinreb amide was then treated with vinyl magnesium bromide to afford the vinyl ketone **17**.

Cross metathesis[19] could now be accomplished between olefins **10–13** and **17** (Scheme 3). Diastereomers **10–13** were each cross-coupled with vinyl ketone **17** by mixing the two intermediates in the presence of a stoichiometric amount of Zhan I catalyst[20] under dilute conditions (0.05 M). Intermediates **18–21** were next converted to the pyrrolidine rings using a catalytic hydrogenation reaction which accomplished five separate reactions in a single pot. Careful tracking of the reaction using LC–MS allowed the sequence of reactions to be identified. Initially, the reduction of the double-bond occurs with 90% conversion after 30 min under 50 PSI hydrogen. Next, removal of the chloride and Cbz protecting groups was observed, followed by formation of the cyclic imine from the ring closure of the ketone with the free amine. Finally, the rate-limiting step in this reaction appears to be the hydrogenation of the imine to form the pyrrolidine ring. The protection of the amines with *tert*-butyl-dicarbonate is the final reaction step to afford the four separate *cis/trans* (3:1 ratio) pyrrolidine diastereomers **26–29** (as shown in Scheme 3).

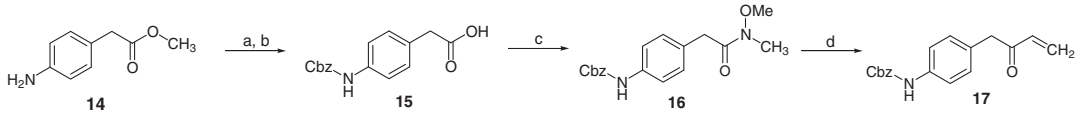
The pyrrolidine scaffolds were now available for synthesis of β_3-AR agonists. We first targeted a sulfonamide analogous to L-796,568. Sulfonyl chloride **30**[21] was reacted with each of the four diastereomers followed by simultaneous deprotection of the amine and hydroxyl groups using 4 N HCl in dioxane with 10% (v/v) water to furnish **31**–**34**.

The acyclic compound (**3**) is also shown to allow comparisons to the pyrrolidine analogs (Table 1). The four diastereomers vary greatly in hβ3-AR agonist potency and hβ1/hβ2-AR selectivity.

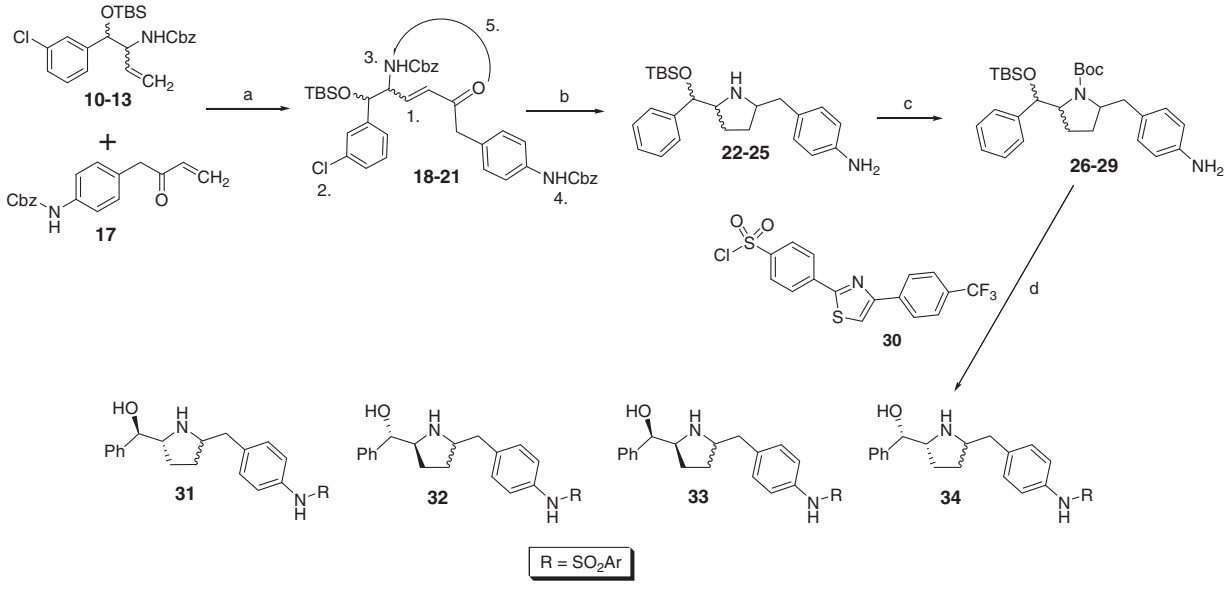
Not surprisingly, analogs with the undesired (*S*)-hydroxyl stereochemistry were 100-fold less potent than the (*R*)-hydroxyl diastereomers. On the other hand, the two analogs with the (*R*)-hydroxyl stereochemistry both exhibited good potency in the hβ3-AR agonist functional assay. Compound **31** was optimal and exhibited similar functional activity as acyclic analog **3**. Since **31** was a 3:1



Scheme 1. Synthesis of ethanolamine intermediates.[16] [a]Reagents and conditions: (a) 1.6 M vinylmagnesium bromide in THF, THF (0–25 °C) 16 h (82%); (b) TBSCl, imidazole, DCM (68%); (c) ozone, DCM (−78 °C) followed by PPh_3 (−78 °C to 25 °C) overnight (71%); (d) *S*-(−)-2-methyl-2-sulfinimide, copper sulfate, DCM, overnight (72%); (e) vinylmagnesium bromide, THF (0 to 25 °C), 5 h (88% total yield); (f) 4 N HCl in dioxane (quantitative); (g) benzyl chloroformate, TEA, DCM (63–82%).



Scheme 2. Synthesis of vinyl ketone intermediate. [a]Reagents and conditions: (a) benzyl chloroformate, TEA, DCM (92%); (b) LiOH, 1:1:1 THF, water, MeOH, 60 °C 5 h (78%); (c) *N*,*O*-dimethyl-hydroxylamine hydrochloride, EDC, HOAt, DMF (69%); (d) 2 equiv vinylmagnesium bromide, THF (−78 °C to 0 °C, 72%).



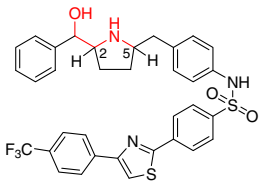
Scheme 3. Synthesis of the pyrrolidine core and sulfonamides **31**–**34**. [a]Reagents and conditions: (a) Zhan I catalyst, DCM, 40 °C 5 h (75%); (b) 10% palladium on carbon, H_2, ethanol (92% total yield); (c) *tert*-butyl di-carbonate, TEA, THF, overnight (83%); (d) 4-{2-[4-(trifluoromethyl)phenyl]-1,3-thiazol-4- yl}benzenesulfonyl chloride, DIEA, DMF, 2 h, rt (62–84%) followed by 4 N HCl in dioxane (10% water v/v), 1 h, rt (88–96%).

mixture of *cis*/*trans* pyrrolidine isomers, separation of the two diastereomers was important to determine the most desired pyrrolidine stereoisomer.

Separation of the *cis* and *trans* pyrrolidine isomers of compound **31** was accomplished by use of preparative SFC using a ChiralCEL OD chiral column eluting with 20% methanol in carbon dioxane as the elutant.[22] Characterization of the major isomer (compound **35**) was done with NOESY ^{1}H NMR which supports an assignment of the relative stereochemistry as a *cis* pyrrolidine.[23]

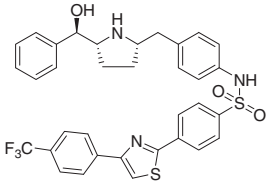
The *cis* pyrrolidine diastereoisomer **35** is 20-fold more potent than the *trans* isomer **36** (Table 2). Additionally, the *cis* diastereomer is more selective over both β_1 and β_2 ARs than the *trans* diastereomer. Further profiling of this compound was halted, however, due to the poor bioavailability observed in rats with compound **35**. After IV dosing, the compound exhibited a clearance of 13.2 mL/min/kg and a half-life of 11.4 h. After oral dosing, however, poor exposures and bioavailability was observed (dose normalized PO AUC = 0.07 μM·h/mpk and oral bioavailability of 3.8%).

Table 1
Sulfonamide analogs

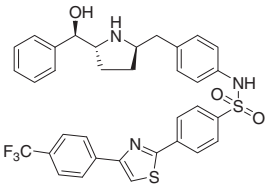


Compound	Chirality		hβ3		Off-target IC_{50}'s (nM)	
	OH	Pyrrolidine	EC_{50} (nM)	Act (%)	hβ1	hβ2
3	*R*	Acyclic	11	95	1040	780
31	*R*	2(*R*), 5(*R*,*S*)	15	101	2930	2180
32	*S*	2(*S*) 5(*R*,*S*)	2030	89	1780	3530
33	*R*	2(*S*), 5(*R*,*S*)	81	104	2150	3530
34	*S*	2(*R*) 5(*R*,*S*)	1307	104	1520	2790

Table 2
Separated diastereomers of compound **31**



35
"cis"



36
"trans"

Compound	EC_{50} (nM)	Act (%)	hβ1	hβ2	CYP3A4	CYP2D6
35	4.1	95	2189	2509	>50,000	>50,000
36	83	76	242	297	>50,000	>50,000

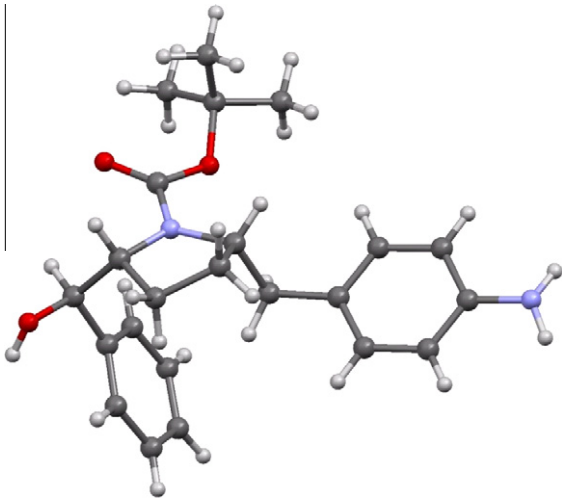
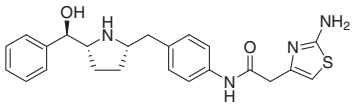


Figure 5. Crystal structure of *cis* pyrrolidine enantiomer (**37**).

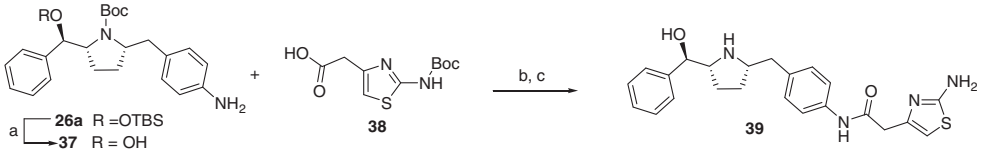
Table 3
Aminothiazole amiole analog (**39**)



Compound 39
EC_{50}: 0.98 nM (99% Act)
hβ1/hβ2 IC_{50}: >20 μM

	Pharmacokinetics		
	Rat	Dog	Rhesus
Clp (ml/min/kg)	11	23	43.4
$t_{1/2}$ (h)	7.7	8.3	9.9
V_d (L/kg)	5.8	14	26.4
PO AUCn (μM·h/mpk)	0.44	0.32	0.14
F (%)	12	18	14

Ancillary activity: CYP inhibition (nM)	Solubility (mg/ml)	Microsomal stability: % remaining (45 min)
2D6 2200	@pH <5.2:>1.8	Dog 23
3A4 >100,000	@pH 7.4:1.3	Human 40
2C9 >100,000	$hNa_v1.5$ 26%I @ 30 μM	Rat 13
2C8 >100,000	hERG IC_{50} 11.2 μM	Rhesus 77



Scheme 4. Synthesis of the pyrrolidine aminothiazole amide. [a]Reagents and conditions: (a) 1.0 M TBAF in THF, rt 4 h (78%, intermediate synthesized for crystallization purposes only); (b) EDC, HOAt, DMF, rt 16 h (83%); (c) 4 N HCl in dioxane with 10% (v/v) water added, rt, 2 h (94%).

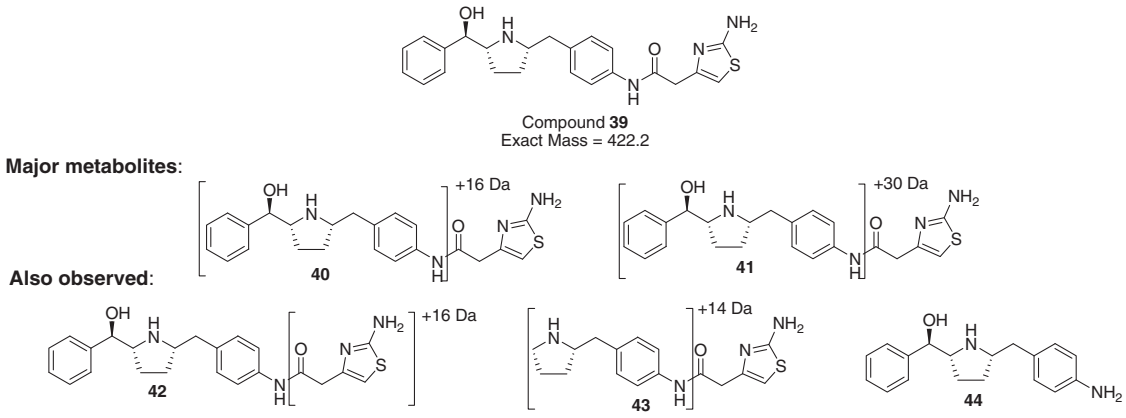


Figure 6. Proposed metabolites observed in human and rat liver microsomes.

After discovering that the *cis* pyrrolidine was the more potent diastereomer, the synthetic route was modified to further improve selectivity for formation of the *cis* diastereomer. Changes in the hydrogenation step in which catalyst loading was decreased to 15% (by weight of catalyst to compound) and dilution increased to a 0.1 M solution, afforded exclusively the *cis* pyrrolidine **26a** with ⩾98% de.[24] The *cis* pyrrolidine stereochemistry was confirmed by X-ray crystallographic analysis of intermediate **37** (Fig. 5) obtained from removal of the *tert*-butyl di-methylsilyl protecting group from intermediate **26a**. Crystallization of intermediate **26a** was attempted but did not yield X-ray quality crystals.

Amide **39** was synthesized using standard EDC coupling procedures followed by acid mediated deprotection (Scheme 4). Compound **39** demonstrated potent in vitro functional human β_3-AR agonist activity with an EC_{50} = 0.98 nM and excellent selectivity over hβ1/hβ2 (>20,000 nM binding affinity).[25] Additionally, promising pharmacokinetic parameters were observed across species with moderate bioavailability (>10%) and half-lives ⩾7.7 h in rats, dogs, and rhesus monkeys. The ancillary activities were acceptable with IC_{50}'s >70 μM observed against CYPs 2C9, 2C8 and 3A4; with the least selectivity seen for CYP2D6 with an IC_{50} observed of 2.2 μM, respectively. Additionally, only moderate inhibition (28% @ 10 μM) of $hNa_v1.5$ and hERG (IC_{50} = 11 μM) was observed (Table 3).[26] Metabolite identification was done with compound **39** after incubation with rat and human liver microsomes over a 45 min period (Fig. 6). The major metabolites observed were oxidation on the aniline core with metabolites identified as having +16 and +30 Daltons (compounds **40** and **41**, respectively) compared to the parent. Additionally, metabolites that correspond to hydrolysis of the aniline amide (**44**) were observed. Metabolites resulting in release of the left hand side or right hand side of **39** were not identified, however, suggesting that the compound indeed would not form similar metabolites to those observed with the previous clinical candidate L-796,568.[15]

In order to assess the risk for mutagenicity, aminothiazole **39** and the aniline metabolite **44** were screened in silico using DEREK.[27] Aniline **44** did not trigger a structure alert by DEREK as a possible genotoxic moiety. On the other hand, aminothiazole **39** was alerted by DEREK as a potential risk for genotoxicity. To support the computational analyses, compounds **39** and **44** were each examined further in mutagenicity assays.

In a 5-fluorouracil forward mutation assay,[28] aminothiazole **39** produced a single positive response in the presence of S-9 metabolic activation, with an increase in adjusted mutants/well that was slightly above the standard deviation recorded for the mean historical control level. This was not considered a strong positive result and was concluded to be equivocal.

To assess the risk of mutagenicity for pyrrolidine aniline core **44**, a full AMES mutagenicity study[29] was performed. Compound **44** was dosed over a concentration range of 30 μg/plate to a maximum concentration of 6000 μg/plate to ensure a robust result. The compound did not produce a 2-fold or greater increase in revertants relative to the control in any of the bacterial strains tested, with or without the S-9 fraction, constituting a negative result in this Microbial Mutagenesis Assay. The negative result supports further research into new β_3-AR agonists by de-risking the core and the possible aniline metabolite for mutagenicity.

In conclusion, we have discovered a novel pyrrolidine core which can be utilized in the exploration of potent and selective human β_3-AR agonists. Sulfonamide **35** and amide **39** derived from the *R,R,S* pyrrolidine core possessed potent human β_3-AR agonist activity with similar or improved selectivity when compared to the clinical candidate L-796,568. Metabolite identification after incubation of compound **39** with rat liver microsomes confirmed that formation of metabolites similar to those observed with L-796,568 was unlikely. Further characterization of compound **39** revealed a risk for genotoxicity due to the aminothiazole functionality that triggered a structure alert in DEREK and an equivocal result from a 5-FU mutagenicity assay. On the other hand, the pyrrolidine aniline core **44** afforded a negative result in AMES assays. Early de-risking of the aniline core has led to further exploration of human β_3-AR agonists that incorporate the pyrrolidine **44** and this research will be reported in the future.

Acknowledgements

The authors would like to thank Karen Dingley and Randy Miller for their support in supplying the metabolite ID data for compound **37** and assistance with the DMPK interpretation. We'd like to acknowledge John DeLuca, Jennifer Smeed, and Gwendolyn Ward for their assistance in the Genotox studies on compounds **39** and **44** which de-risked the pyrrolidine aniline core for our future endeavors in the discovery of potent β_3-AR agonists. One final thanks goes out to Terry Butler for her guidance and helpful conversations which encourage the completion of this manuscript.

Supplementary data

Supplementary data (structures, experimental, and characterization data for all compounds including X-ray crystal structure of compound **37** (CCDC 787767), NOE and spectral data for compound **35** and HPLC trace of the separation of compounds **35** and **36** is available free of charge via the Internet at http://pubs.acs.org)

associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.087.

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21. This material was obtained from prior batches synthesized for the scale-up of L-796,568. The chemistry to prepare this intermediate has been document previously.[10]
22. The major isomer eluted first at retention time of 4.11 min, while the second isomer eluted at 4.56 min.
23. The stereochemistry of the two pyrrolidine chiral centers was determined to be *cis* based on 2D NOE NMR data (see Supplementary data).
24. Selectivity was assessed by SFC analytical analysis and the effort to improve selectivity was studied via trial and error of changes in the reaction conditions. Original less selective conditions: Dilution 0.02 M and stoichiometric amount (by weight) of the catalyst (resulted in 3:1 mixture of *cis*/*trans*).

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