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# 4-Aminopiperidine Ureas as Potent Selective Agonists of the Human $\beta_3$-Adrenergic Receptor

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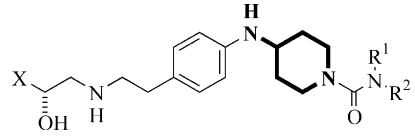
**Abstract**—The preparation and structure–activity relationships (SARs) of potent agonists of the human $\beta_3$-adrenergic receptor (AR) derived from a 4-aminopiperidine scaffold are described. Examples combine human $\beta_3$-AR potency with selectivity over human $\beta_1$-AR and/or human $\beta_2$-AR agonism. Compound **29s** was identified as a potent ($EC_{50} = 1$ nM) and selective (greater than 400-fold over $\beta_1$- with no $\beta_2$-AR agonism) full $\beta_3$-AR agonist with in vivo activity in a transgenic mouse model of thermogenesis.


β-Adrenoceptors (β-ARs) were subclassified as $\beta_1$-AR and $\beta_2$-AR in 1967. Increased heart rate is the primary consequence of $\beta_1$-AR stimulation, while bronchodilation and smooth muscle relaxation typically result from $\beta_2$-AR stimulation. Adipocyte lipolysis (breakdown of fat) was initially thought to be solely a $\beta_1$-AR mediated process. However, results from the early 1980s indicated that the receptor mediating lipolysis was atypical in nature. These atypical receptors were designated $\beta_3$-ARs and are found on the cell surface of both white and brown adipocytes where their stimulation promotes both lipolysis and energy expenditure.[1] More recently the $\beta_3$-AR has been shown to play a role in the relaxation of human urinary bladder detrusor tissue.[2] Early compounds with greater agonist activity for the stimulation of lipolysis ($\beta_3$-AR) than for the stimulation of atrial rate ($\beta_1$-AR) and tracheal relaxation ($\beta_2$-AR) showed potential as anti-obesity and anti-diabetic agents in rodents.[3]

Disappointing human clinical trials with one such agent (CL-316243, BTA-243), optimized for the rodent $\beta_3$-AR, have been attributed to partial rather than full agonism at the human $\beta_3$-AR and poor oral bioavailability.[4] A new phase of $\beta_3$-AR agonist discovery at Wyeth-Ayerst Research thus utilized a Chinese hamster ovary (CHO) cell line expressing the cloned human $\beta_3$-AR receptor[5] in addition to the human $\beta_1$- and $\beta_2$-ARs. In vivo activity was assessed using a transgenic mouse model of thermogenesis.[6]

This paper describes a systematic approach undertaken to explore the utility of the 4-piperidine scaffold (Fig. 1, Structures Ia and Ib) in the design of human $\beta_3$-AR agonists.




Ia = Ar = Phenethylamines
Ib = $ArOCH_2$- = Phenoxypropanolamines

**Figure 1.** Urea derivatized 4-aminopiperidine template for human $\beta_3$-AR agonism.

Division of the molecule into two pieces on either side of the secondary alkyl amine suggested either asymmetric epoxides such as **4** or asymmetric β-amino alcohols such as **23** as key structural building blocks. Routes to these are shown in Schemes 1–3. Mitsunobu reaction of commercial *R*-(+)-glycidol with phenols **3**[7] and **7** (Scheme 1) provided epoxides **4** and **8**,[8] respectively. Other more elaborate epoxides (e.g., **11**[9] and **14**[10]) (Scheme 2) were provided by alkylation of the corresponding phenols

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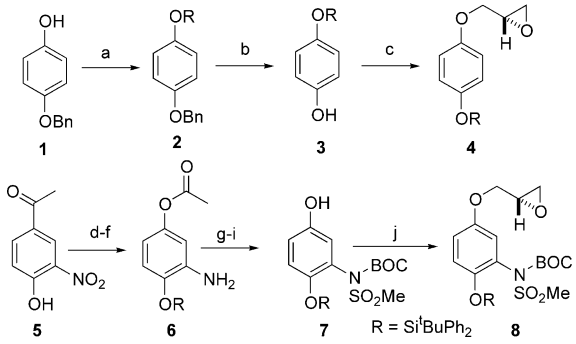


**Scheme 1.** (a) $^{t}BuPh_2SiCl$, imidazole, $CH_2Cl_2$, 82%; (b) Pd/C, cyclohexene, EtOH, 94%; (c) *R*-(+)-glycidol, $PPh_3$, DEAD, THF, 56%; (d) $^{t}BuPh_2SiCl$, imidazole, $CH_2Cl_2$, 98%; (e) MCPBA, $CHCl_3$, 85%; (f) Raney Ni, $H_2$, THF, 73%; (g) mesyl chloride, THF, $^{i}Pr_2NEt$, 66%; (h) $Boc_2O$, $CH_2Cl_2$, 96%; (i) NaOH, MeOH, 79%; (j) *R*-(+)-glycidol, DEAD, $PPh_3$, 70%.

with the commercially available (2*S*)-(+)-glycidyl-3-nitrobenzenesulfonate.

In the case of epoxide **17**, an alternate synthesis to that previously disclosed[11] was developed starting from trisubstituted phenol **15**. Here the alkylation with (2*S*)-(+)-glycidyl-3-nitrobenzenesulfonate was performed prior to the reduction of nitroaniline **16** and ring closure with phosgene yielding **17** (Scheme 2).

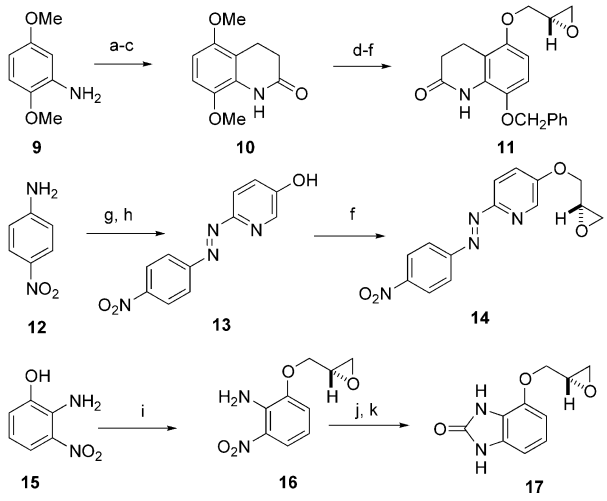
Alternatively, when phenethylamines were the target agonists epoxides such as **20** were constructed or purchased [(*R*)-(+)-3-chlorostyrene oxide for the preparation of **37a**]. An alternative strategy required chiral amino alcohol **23**, and this was prepared as shown in Scheme 3.

The preparation of the scaffold piperidine **27** is shown in Scheme 4. Selective protection of the primary amine of **24** as the BOC amide was followed by reductive amination with 4-benzylpiperidinone to yield **26**. Removal of the benzyl protecting group gave the secondary amine **27**.

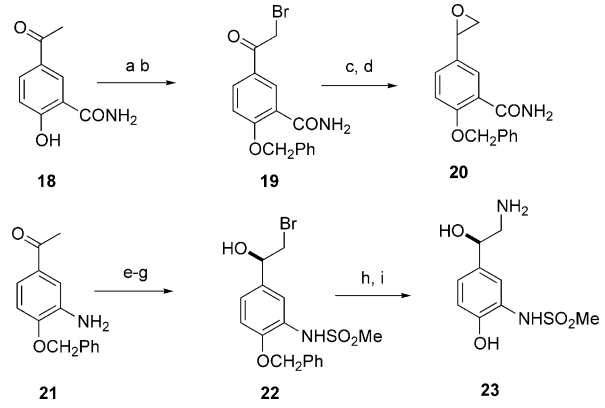
Reaction of **27** with isocyanates or with triphosgene followed by an amine provided ureas **28** depending on reagent availability. Following purification and removal of the BOC group with formic acid the formate salt **28** was used directly or treated with NaOH to liberate the free base.

Ureas **35** proved more difficult to prepare. The instability of the intermediate nitro aldehyde, generated by oxidation of **30**, could be avoided if the Dess–Martin oxidation was followed immediately by protection as the dimethyl acetal to give **31** as shown in Scheme 5. Reduction of the nitro group of **31** provided aniline **32**. Ureas **35** were obtained from **32** as described for **27** above.

Intermediate ureas **28** were reacted directly with epoxides in the presence of a hindered organic base, thus providing access to either aryloxypropanolamines or phenethylamines as shown in Scheme 6. This reaction was not regiospecific and access to the starting epoxides was not always feasible. An alternative approach is illustrated in the preparation of **37c**. Here, in situ gen-



**Scheme 2.** (a) β-Ethoxyacryl chloride, $Et_3N$, benzene, 99%; (b) HCl, 78%; (c) Raney Ni, $H_2$ $^{i}PrOH$, THF, 47%; (d) 48% HBr, heat, 80%; (e) acetone, water, $K_2CO_3$, $PhCH_2Br$, 27%; (f) $K_2CO_3$, 2-butanone, (2*S*)-(+)-glycidyl-3-nitrobenzenesulfonate, 82 and 32% for **13** to **14**; (g) $NaNO_2$, $H_2O$, HCl (concd); (h) NaOH, 3-hydroxypyridine, 22% steps g and h; (i) $K_2CO_3$, acetone, (2*S*)-(+)-glycidyl-3-nitrobenzenesulfonate, 28%; (j) Raney Ni, ethanol; (k) phosgene, $CH_2Cl_2$, 51% for steps j and k.

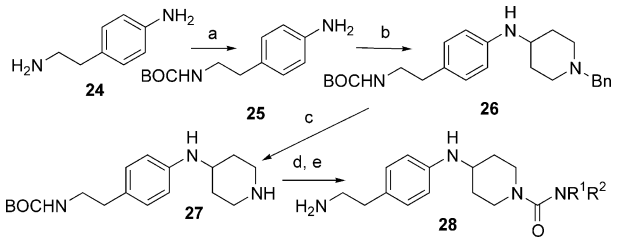


**Scheme 3.** (a) $PhCH_2Br$, NaOMe, MeOH, 80%; (b) $Br_2$, $CHCl_3$, 87%; (c) $NaBH_4$, ethanol, THF, 96%; (d) $K_2CO_3$, 2-butanone, 74%; (e) $MeSO_2Cl$, pyridine, $CH_2Cl_2$, 47%; (f) $CuBr_2$, $CHCl_3$, 57%; (g) (*R*)-2-methyl-CBS-oxazaborolidine, $BH_3$, THF, 61%; (h) $NaN_3$, DMSO; (i) $H_2$, Pd/C, MeOH, 71% for steps h and i.

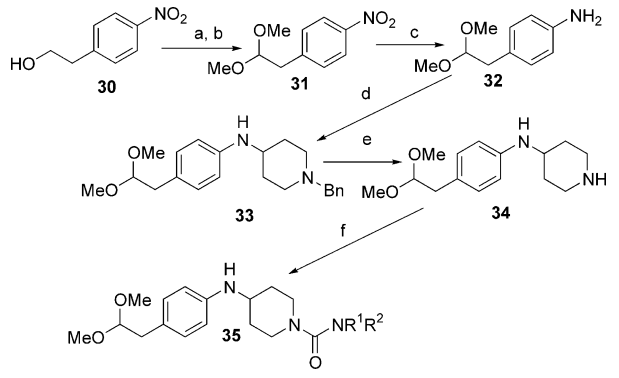
eration of the aldehyde **38** and subsequent reductive amination provided phenethylamines such as **37c**.[12]

The in vitro data for a selection of simple alkyl ureas based on the 4-aminopiperidine scaffold is presented in Table 1. In general molecules of this type (**29a**–**f**) have comparable human $\beta_3$-AR agonism relative to the standard isoproterenol, considerably weaker $\beta_2$-AR agonism and some selectivity over $\beta_1$-AR agonism. The greatest receptor subtype selectivity is found when the alkyl group is an extended straight chain (cf. **29a** and **29b**). Importantly the potency of $\beta_1$-AR agonism appears to fall off as the chain length increases.

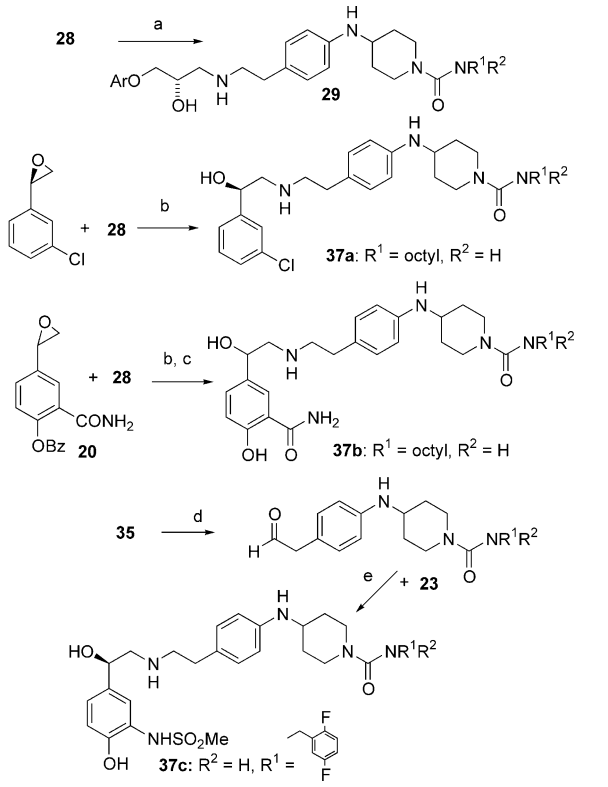
An extensive selection of aryloxypropanolamines was prepared holding the alkyl urea portion of **29a** constant. The importance of the 4-hydroxy group of **29a** was demonstrated by its removal to give **29g** which is an



**Scheme 4.** (a) $BOC_2O$, $CH_2Cl_2$, 92%; (b) 4-benzylpiperidinone, $Na(OAc)_3BH$, AcOH, $Na_2SO_4$, 90%; (c) Pd/C, cyclohexene, ethanol, 96%; (d) isocyanate, THF (75–90%) or amine, triphosgene, $CH_2Cl_2$, (50–80%); (e) formic acid (80–95%).



**Scheme 5.** (a) Dess–Martin oxidation, $CH_2Cl_2$; (b) trimethyl orthoformate, PTSA, methanol, 89% for steps a and b; (c) Pd/C, ammonium formate, ethanol, 76%; (d) 4-benzylpiperidinone, $Na(OAc)_3BH$, AcOH, $Na_2SO_4$, 90%; (e) isocyanate, THF (75–90%) or amine, triphosgene, $CH_2Cl_2$, (50–80%); (f) formic acid, (80–95%).



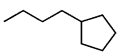
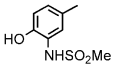
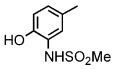
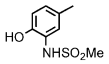
**Scheme 6.** (a) Aryloxy epoxide, ethanol, $^{i}Pr_2NEt$, heat (30–75%); (b) ethanol, $^{i}Pr_2NEt$, heat, 16%; (c) $H_2$ Pd/C, ethanol, 5% for steps b and c (20–37%); (d) NaI, $MeSiCl_3$, $CH_3CN$; (e) MeOH, $NaCNBH_3$, AcOH, 7% for steps d and e.

**Table 1.** Agonist activity at cloned human β-ARs of alkyl urea substituted piperidines

| Compd | Ar | $R^1$ | $R^2$ | $\beta_3$ $EC_{50}$ (nM) IA (%)[a] | $\beta_1$ $EC_{50}$ (nM) IA (%)[a] | β $EC_{50}$ (nM) IA (%)[a] |
|---|---|---|---|---|---|---|
| **29a** | 4-OHPh | Octyl | H | 8 (>100) | 610 (53) | ia[c] |
| **29b** | 4-OHPh | Me | H | 47 (85) | 350 (106) | ia[c] |
| **29c** | 4-OHPh | H | H | 30 (100) | 511 (77) | ia[c] |
| **29d** | 4-OHPh | $^{i}$Pr | H | 90 (105) | 943 (54) | ia[c] |
| **29e** | 4-OHPh | Chexl | H | 80 (112) | 85 (68) | 77 (17) |
| **29f** | 4-OHPh | Et | ethyl | 36 (96) | 164 (74) | ia[c] |
| **29g** | 4-FPh | Octyl | H | 37%@10 μM | nd[b] | nd[b] |
| **29h** |  | Octyl | H | 290 (68) | nd[b] | nd[b] |
| **29i** |  | Octyl | H | ia@0.1μM | nd[b] | nd[b] |
| **29j** |  | Octyl | H | 66 (67) | 88 (60) | nd[b] (16) |
| **37a** | 3-ClPh | Octyl | H | 1020 (77) | nd[b] | nd[b] |
| **37b** (racemic) |  | Octyl | H | 42%@10 μM | nd[b] | nd[b] |

[a]β-ARs agonistic activities were assessed by measurement of cAMP accumulation levels in CHO cells expressing human β-ARs; the intrinsic activities (IA) are given as a percentage of maximal stimulation relative to isoproterenol.
[b]nd, not determined.
[c]ia, inactive.

**Table 2.** Agonist activity at cloned human β-ARs of urea substituted piperidines

| Compd | Ar | $R^1$ | $R^2$ | $\beta_3$ $EC_{50}$ (nM) IA (%)[a] | $\beta_1$ $EC_{50}$ (nM) IA (%)[a] | $\beta_2$ $EC_{50}$ (nM) IA (%) |
|---|---|---|---|---|---|---|
| **29k** | 4-OHPh |  | H | 50 (90) | 187 (27) | ia[c] |
| **29l** | 4-OHPh | 4-Fbenzyl | H | 41 (93) | 109 (63) | ia[c] |
| **29m** | 4-OHPh | 2,4-diFbenzyl | H | 30 (84) | 460 (106) | ia[c] |
| **29n** | 4-OHPh | 2,4-diClbenzyl | H | 250 (91) | 254 (56) | ia[c] |
| **29o** | 4-OHPh | 2-Fbenzyl | H | 37 (91) | 260 (36) | ia[c] |
| **29p** | 4-OHPh | 2,6-diFbenzyl | H | 32 (104) | 750 (75) | ia[c] |
| **29q** | 4-OHPh | 2,5-diFbenzyl | H | 23 (92) | 522 (48) | ia[c] |
| **29r** |  | Octyl | H | 200 (99) | nd[b] (22) | ia[c] |
| **29s** |  | 2,5-diFbenzyl | H | 1 (100) | 423 (75) | ia[c] |
| **37c** |  | 2,5-diFbenzyl | H | 5 (100) | 2640 (78) | 10 (110) |

[a]See footnotes for Table 1.
[b]See footnotes for Table 1.
[c]See footnotes for Table 1.

essentially inactive compound. Similarly more complex replacements (**29h–j**) also significantly reduced activity. Phenethylamines (**37a,b**) were prepared but offered no improvement.

The induction of thermogenesis in human $\beta_3$-AR transgenic mice (Tg mice) by **29a** was compared with its ability to induce thermogenesis in $\beta_3$-AR knock-out mice (KO mice). This assay was employed as an in vivo measure of the potential of these agonists to treat or inhibit disorders related to obesity or type II diabetes. Disappointingly **29a** had weak in vivo activity in the human $\beta_3$-AR transgenic mouse ($11\% \pm 8\%$ at 10 mg/kg ip). One possible reason for the low in vivo activity was suggested by Phase I metabolism studies using isolated P450 rat microsomes. It was demonstrated in this assay that the long alkyl chain was a site of oxidation. A number of capped alkyl ureas were prepared in order to overcome this liability. In the case of the cyclopentylpropyl urea **29k** the $\beta_3$-AR agonism was reduced compared to **29a** and no improvement in in vivo activity was observed.

A more successful approach is illustrated with **29l**. Here a robust and selective in vivo response ($46\% \pm 4\%$ at 10 mg/kg ip) was measured. However, there has been a decrease in both $\beta_3$-AR agonism and selectivity. Systematic variations of the substituents on the phenyl ring led to the identification of **29q** (Table 2). Here the 2,5-di fluoro substituent pattern provided a $\beta_3$-AR agonist with over 20-fold selectivity against $\beta_1$-AR partial agonism and devoid of $\beta_2$-AR agonism.

Further Phase I metabolic studies highlighted the 4-hydroxyphenyloxy portion of the molecule as a potential liability and a further round of optimization was undertaken. Firstly, comparing **29a** with **29r** it is clear that there is a significant loss of activity when the $NHSO_2Me$ substituent was introduced adjacent to the phenol. Importantly, switching to the 2,5-difluoro capped urea and the corresponding phenethylamine provided the potent $\beta_3$-AR agonist **37c**. However, little selectivity over $\beta_2$-AR agonism was observed.

Significantly, when the ethanolamine portion of **37c** was replaced with the corresponding aryloxypropanolamine to give **29s**, potent $\beta_3$-AR agonism ($EC_{50} = 1$ nM) was combined with high selectivity over both $\beta_1$-AR and $\beta_2$-AR. Compound **29s** was also demonstrated to be a selective human $\beta_3$-AR agonist in vivo ($22\% \pm 7\%$ at 10 mg/kg ip) and thus shows promise as an agent for the treatment of obesity, type II diabetes and frequent urination.

In conclusion 4-aminopiperidine has been demonstrated to be a viable scaffold for the preparation of human $\beta_3$-AR agonists. Optimization of piperidine alkyl ureas derived from this structural element led to the identification of many in vitro selective $\beta_3$-AR agonists. In vivo results in a $\beta_3$ transgenic mouse of thermogenesis and supporting data from in vitro P450 microsomal studies led to the identification of aromatic capped ureas. The selectivity of these aromatic capped ureas was improved by varying the substituents and substitution pattern around the phenyl ring. The resulting **29s** was found to be a potent human $\beta_3$-AR full agonist ($EC_{50} = 1$ nM) with greater than 400-fold selectivity over $\beta_1$-AR and no $\beta_2$-AR agonism. In addition **29s** was active at a dose of 10 mg/kg in the acute in vivo $\beta_3$ transgenic mouse model of thermogenesis.

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