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Human β_3 Adrenergic Receptor Agonists Containing Cyanoguanidine and Nitroethylenediamine Moieties

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Abstract—Pyridineethanolamine derivatives containing cyanoguanidine or nitroethylenediamine moieties were examined as human β_3 adrenergic receptor (AR) agonists. Notably, indoline derivatives **6a** and **11** were potent β_3 AR agonists (β_3 $EC_{50} = 13$ and 19 nM, respectively), which showed good selectivity over binding to and minimal activation of the β_1 and β_2 ARs.

Elevation of metabolic rate by activation of the human β_3 adrenergic receptor (AR) may be an effective approach toward the treatment of obesity.[1] Heretofore, our efforts have largely focused on the preparation of analogues containing a benzenesulfonamide functionality, exemplified by indoline **1** (Fig. 1).[2] This compound is a potent β_3 AR agonist ($EC_{50} = 0.93$ nM), which shows >1000-fold selectivity over binding to the β_1 and β_2 ARs. This excellent in vitro profile is due in part to the presence of the benzenesulfonamide moiety.

While examining potential replacements of the benzenesulfonamide in an attempt to improve the overall in vivo properties of these compounds, we investigated a series of thiourea derivatives. Interestingly, the compounds were potent agonists of the β_3 AR (data not shown). Indoline **2**, for example, showed only a 6-fold loss in potency at the β_3 AR compared to sulfonamide **1** and was somewhat selective over binding to the β_1 and β_2 ARs (20- and 50-fold, respectively).[3]

The thiourea moiety is prone to in vivo metabolism that can result in significant toxicity.[4] We decided, therefore, to focus our investigation on the bioisosteric cyanoguanidine and nitroethylenediamine moieties. Herein we would like to report the results of this study that not only included the preparation of simple aryl cyanoguanidines, but was extended to more fully explore a series of indolines related to analogues **1** and **2**. Both the 4-octyl thiazole and a 4-(4-trifluoromethoxy)phenyl thiazole indoline were included. Three positions of attachment of the cyanoguanidine to the indoline ring were also investigated. This work resulted in the discovery of a novel series of potent, selective human β_3 AR agonists containing a cyanoguanidine moiety. Preliminary data of simple nitroethylenediamine analogues will also be reported.[5]

Cyanoguanidines **3**–**6** and nitroethylenediamines **7** were prepared from aniline **8**[6] by reaction with either diphenyl cyanocarbonimidate in acetonitrile or 1,1-bis(methylthio)-2-nitroethylene in isopropanol (Scheme 1).[7] This was followed by displacement with the amine and deprotection to yield the desired compounds **3**–**7**. The requisite indoline derived amines **9** were prepared from the corresponding nitroindoline[8] by reaction with potassium thiocyanate to yield thiourea **10**. Condensation with a chloroketone and reduction with stannous chloride yielded anilines **9**.

Cyanoguanidines **3** were tested at the human β ARs and the results are shown in Table 1. The compounds **3a**–**h** were partial agonists of the β_3 AR and were generally only moderately potent (β_3 $EC_{50} = 26$–150 nM). Comparison of iodo derivatives **3a** and **3b** showed a slight preference for *meta* substitution. The selectivity for the β_3 AR, however, was generally very low. Only the 1-naphthyl derivative **3h** showed >10-fold selectivity over binding to both the β_1 and β_2 ARs. Agonist activity at the β_1 and β_2 ARs was not measured.

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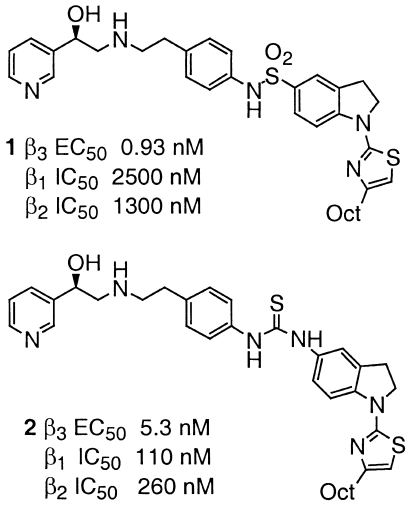


Figure 1.

In an effort to improve the in vitro profile of the cyanoguanidine β_3 AR agonists, we investigated indoline derivatives **4**–**6** (Table 2). The in vitro results showed that activity and selectivity of these compounds were highly dependent on the position of the cyanoguanidine moiety on the indoline ring. The 5-substituted compounds **4**, which had traditionally been preferred in the sulfonamide series,[9] were potent human β_3 AR agonists (β_3 $EC_{50} = 11$ and 13 nM). Both these derivatives, however, lacked any selectivity over binding to the β_1 and β_2 ARs. Additionally, compound **4b** was a full agonist of the β_1 AR. Substitution at the 6-position was not preferred, as octyl derivative **5a** showed a 5-fold loss in potency when compared to its isomer **4a**, and the aryl analogue **5b** did not activate the β_3 AR at 1 μM.

The 4-substituted indolines **6** proved to have a much more interesting overall in vitro profile. These compounds were partial agonists of the β_3 AR (62 and 72% of the maximal response of isoproterenol), with only minimal activation of the β_1 or β_2 AR at 10 μM. Octyl analogue **6a** was a 13 nM β_3 AR agonist which exhibited >70-fold selectivity over binding to both the β_1 and β_2 ARs. The 4-trifluoromethoxyphenyl compound **6b** was slightly less potent but displayed improved selectivity over binding to the β_1 and β_2 ARs (>140- and >175-fold, respectively).

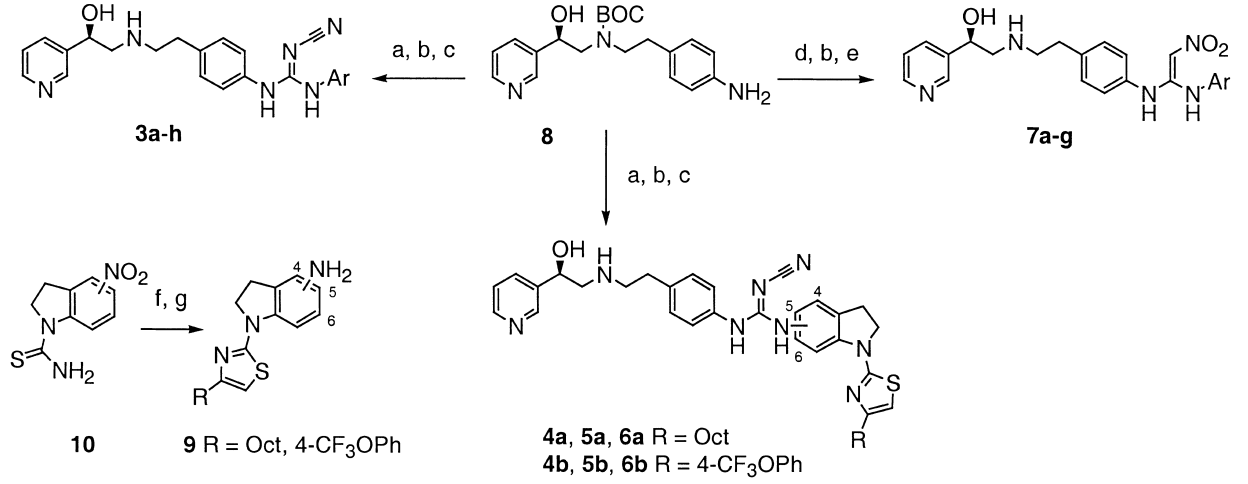
Table 1. Activity of cyanoguanidines **3** at the cloned human β adrenergic receptors

Compd	Ar	β_3 EC_{50} nM (%act)[a]	β_1 binding IC_{50}[b] nM	β_2 binding IC_{50}[b] nM
3a	4-I-Ph	100 (38)	730	170
3b	3-I-Ph	59 (51)	230	67
3c	3,5-DichloroPh	89 (72)	290	140
3d	3-F-Ph	100 (69)	590	200
3e	3-$CONH_2$Ph	150 (76)	980	400
3f	3-PhOPh	26 (70)	240	40
3g	2-Naphthyl	130 (41)	740	50
3h	1-Naphthyl	42 (64)	480	570

[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.

An impressive improvement in selectivity could also be achieved in the 5-substituted series by modification of the central portion of the molecule. Replacement of the phenyl ring with a naphthyl group had been explored in the benzenesulfonamide series but yielded less potent β_3 AR agonists.[10] This modification was incorporated into cyanoguanidine **4b** to give compound **11** (Fig. 2, Table 2), and in this case there was no significant loss in potency at the β_3 AR ($EC_{50} = 19$ nM). Naphthalene **11** did not activate the β_1 and β_2 ARs to any extent at 10 μM. This was a major improvement over phenyl derivative **4b**, which had significant agonist activity at the β_1 AR. Selectivity over binding to the β_1 and β_2 ARs also increased dramatically from 5- and 13-fold, respectively, for compound **4b**, to 120- and 350-fold, respectively, for the naphthyl analogue.

Simple aryl analogues were also prepared in the nitroethylenediamine series **7** and the initial results were very promising (Table 3). Unlike cyanoguanidines **3**, these



Scheme 1. (a) $NCN{=}C(OC_6H_5)_2$, CH_3CN, 81 °C; (b) $ArNH_2$ or **9**, 2-PrOH, 100 °C, sealed tube; (c) HCl(g)/Et_2O; (d) $O_2NCH{=}C(SCH_3)_2$, 2-PrOH, 95 °C, sealed tube; (e) TFA, CH_2Cl_2; (f) $RCOCH_2Cl$, EtOH, 78 °C; (g) $SnCl_2$, EtOH, 70 °C.

Table 2. Activity of indoline derived cyanoguanidines **4–6** and **11** at the cloned human β adrenergic receptors

Compd	Position of subn on indoline ring	R	β_3 EC_{50} nM (% act)[a]	β_1 EC_{50} nM (% act)[a]	β_1 binding IC_{50}[b] nM	β_2 EC_{50} nM (% act)[a]	β_2 binding IC_{50}[b] nM
4a	5	*n*-Oct	11 (39)	nd[c]	16	nd[c]	50
4b	5	4-CF_3OPh	13 (82)	41 (73)	65	(51@10,000)	180
5a	6	*n*-Oct	59 (38)	nd[c]	74	nd[c]	66
5b	6	4-CF_3OPh	(11@1000)	(27@10,000)	620	(20@10,000)	710
6a	4	*n*-Oct	13 (62)	(11@10,000)	1200	(25@10,000)	930
6b	4	4-CF_3OPh	57 (72)	(6@10,000)	8400	(20@10,000)	>10,000
11	5	4-CF_3OPh	19 (62)	(2@10,000)	2300	(11@10,000)	7100

[a]Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol. Single point data are reported in parentheses as (% activation@concentration in nM).
[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]nd, not determined.

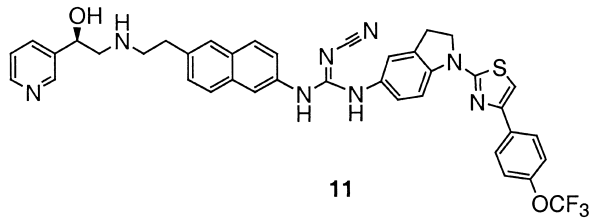


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Figure 2.

Table 3. Activity of nitroethylenediamines **7** at the cloned human β adrenergic receptors

Compd	Ar	β_3 EC nM (% act)[a]	β_1 binding IC_{50}[b] nM	β_2 binding IC_{50}[b] nM
7a	3-I-Ph	22 (97)	1100	1200
7b	3,5-DichloroPh	35 (85)	3800	5600
7c	3-F-Ph	300 (79)	3000	2400
7d	3-$CONH_2$Ph	10 (101)	790	1200
7e	3-PhOPh	120 (93)	830	220
7f	2-Naphthyl	180 (74)	820	3400
7g	1-Naphthyl	140 (79)	1800	370

[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.

derivatives were full agonists of the β_3 AR (74–101% of the maximal response of isoproterenol) and those compounds tested had minimal agonist activity at the β_1 and β_2 ARs (e.g., **7a** and **7b** caused only 11–21% of the maximal response of isopretorenol, data not shown). Compounds **7a**, **7b**, and **7d** exhibited moderate to good potency at the β_3 AR and a much greater degree of selectivity over the β_1 and β_2 ARs than had been seen with the analogous cyanoguanidines (cf Table 1). For example, carboxamide **7d** and 3,5-dichlorophenyl derivative **7b** were >70- and >100-fold selective for the β_3 AR, respectively.

In this paper we have described a novel series of β_3 AR agonists in which the sulfonamide moiety has been replaced with a cyanoguanidine. SAR studies highlighted the different structural preferences in this series which resulted in the discovery of potent, selective β_3 AR agonists. In particular, the 4-substituted indolines **6** exhibit very little activation of the β_1 and β_2 ARs and >70-fold selectivity for the β_3 AR over binding to the β_1 and β_2 ARs. Replacement of the central phenyl ring with a naphthyl group gave compound **11**, which shows a greatly improved in vitro profile over its phenyl analogue **4b**. Finally, preliminary data has shown that a series of simple nitroethylenediamines **7** are potent, full agonists of the β_1 AR, which exhibit up to 100-fold selectivity over binding to the β_1 and β_2 ARs. Both these series represent an important development in the discovery of non-sulfonamide pyridineethanolamine human β_3 AR agonists.

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