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POTENT, SELECTIVE HUMAN β3 ADRENERGIC RECEPTOR AGONISTS CONTAINING A SUBSTITUTED INDOLINE-5-SULFONAMIDE PHARMACOPHORE

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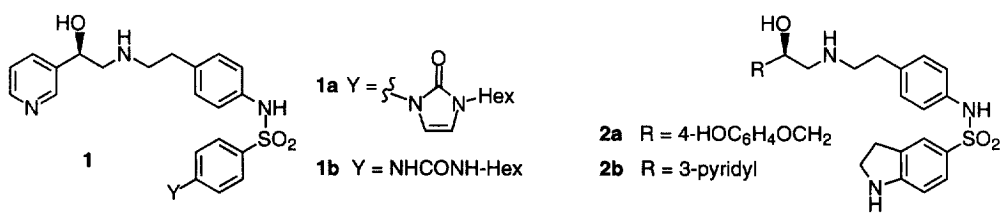
Abstract: A series of compounds possessing an N-substituted indoline-5-sulfonamide pharmacophore was prepared and evaluated for their human β3 adrenergic receptor agonist activity. The SAR of a wide range of urea and heterocyclic substituents is discussed. 4-Octyl thiazole compound **8c** was the most potent and selective compound in the series, with 2800-fold selectivity over β1 binding and 1400-fold selectivity over β2 binding.

Introduction: In recent years it has been well established in several mammalian species that β3 adrenoceptors play a major role in regulating lipolysis and thermogenesis in adipose tissue.[2] As such, the development of specific agents capable of increasing metabolic rate by selective activation of the β3 adrenoceptor constitutes a potentially effective approach for the treatment of obesity, and the utility of a number of β3 adrenergic receptor agonists for this purpose has been investigated.[3] As part of an ongoing program to discover compounds which act as selective agonists at the human β3 adrenoceptor, we have identified a class of aryl sulfonamides possessing the general structure **1** that display significant β3 agonist activity and exhibit minimal cross-reactivity at the β1 and β2 receptors.[4]



SAR studies revealed that maximal potency and selectivity was observed with those compounds possessing substitution at the 4-position of the arylsulfonamide moiety, and within this structural class it was found that incorporation of a cyclic (**1a**; EC_{50} 14 nM) or acyclic (**1b**; EC_{50} 6.3 nM) urea functionality resulted in enhanced potency and selectivity.[4a] Previously we had found that incorporation of an unsubstituted indoline-5-sulfonamide into a series of aryloxypropanolamine analogs (e.g., **2a**; EC_{50} 0.65 nM) afforded a number of potent β3 agonists. Although the unsubstituted indoline-5-sulfonamide **2b** in the pyridylethanolamine series was found to be only a weak partial agonist at the human β3 receptor, herein we wish to report that the combination of a

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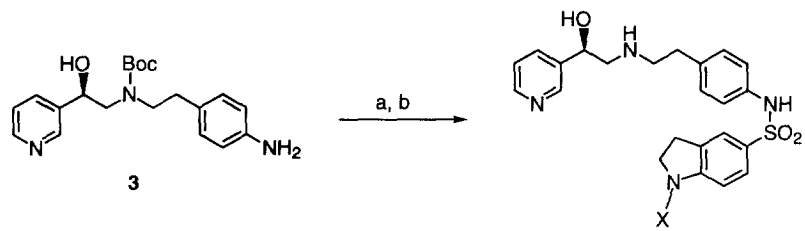
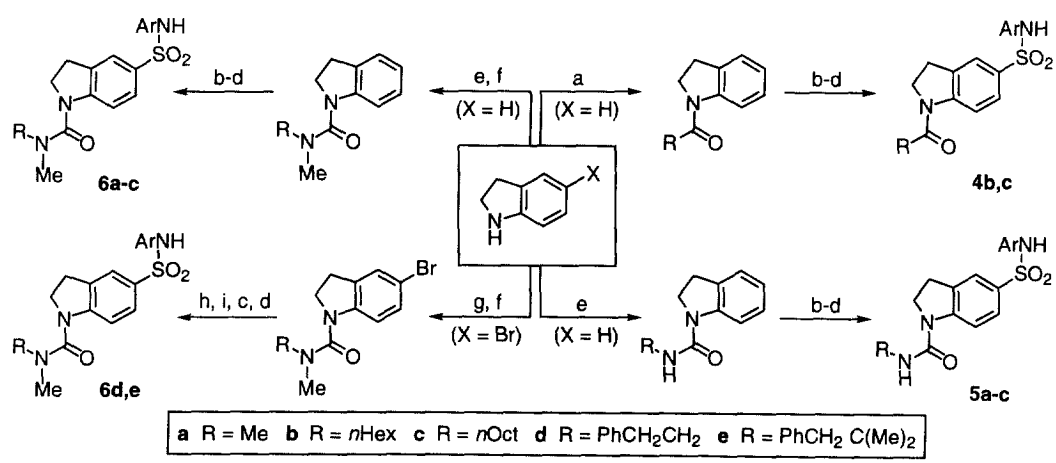
variety of urea or heterocyclic functionalities with the indoline-5-sulfonamide pharmacophore and the 3-pyridyl-ethanolamine moiety of **1** results in a structurally distinct and promising class of β_3 adrenoceptor agonists, many of which exhibit significantly enhanced potency while still retaining selectivity over β_1 and β_2 binding.

Synthesis: The synthetic route utilized to prepare the indoline sulfonamide compounds is illustrated in Scheme 1. Condensation of aniline **3**[4a] with the requisite sulfonyl chloride followed by removal of the BOC protecting group afforded the desired benzenesulfonamides.



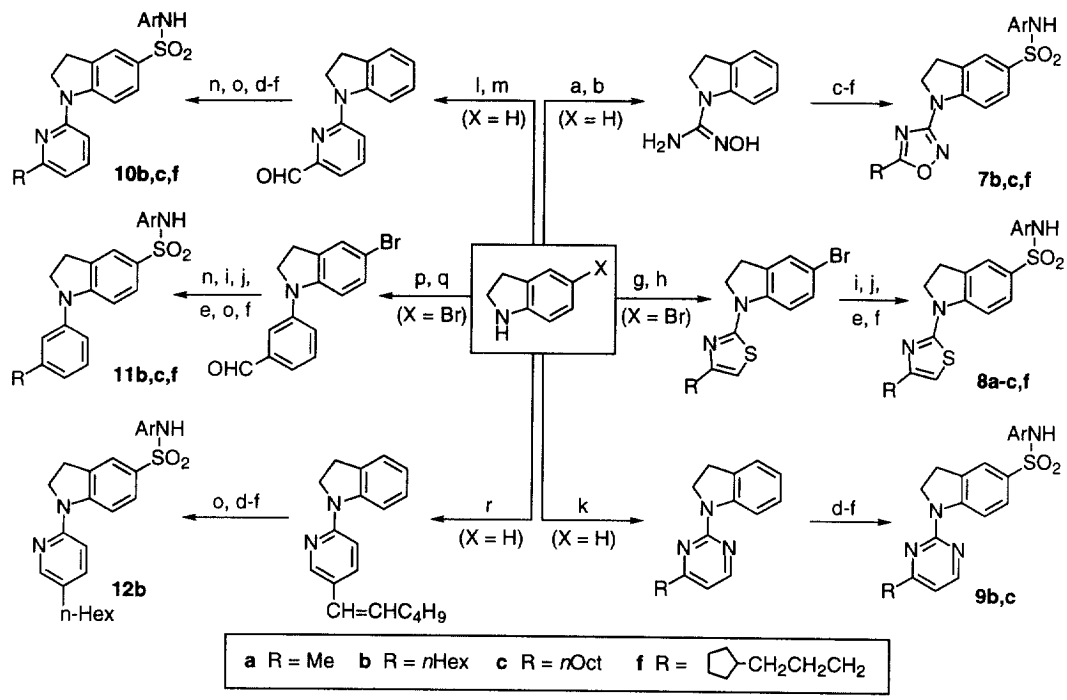
Scheme 1. *Reagents:* (a) $ArSO_2Cl$, pyridine, CH_2Cl_2, 25 °C; (b) TFA-CH_2Cl_2, 25 °C.

The indoline-5-sulfonyl chlorides were generally prepared by either direct chlorosulfonation of the corresponding arenes, or from the aryl bromide by a two step procedure[5] involving oxidative chlorination of an intermediate lithium sulfinate. The required arenes and aryl bromides were, in turn, obtained by standard synthetic procedures as detailed below. Thus, acylation or condensation of indoline with commercially available isocyanates followed by chlorosulfonation and condensation with aniline afforded the N-acyl compounds **4** and N-urea compounds **5** (Scheme 2). N-Methylation prior to chlorosulfonation afforded **6a–c**, while the alternate sulfonation route was employed for the preparation of **6d–e**.



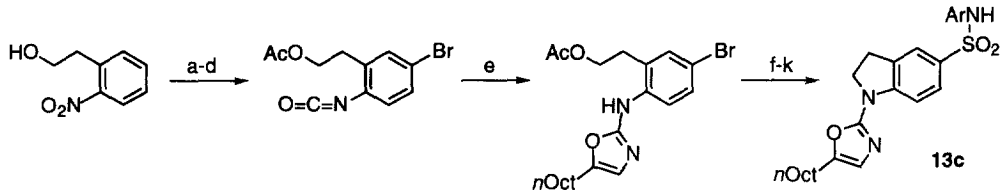
Scheme 2. *Reagents:* (a) RCOCl, pyr, 25 °C; (b) $ClSO_3H$, 0 °C; (c) **3**, pyridine, CH_2Cl_2, 25 °C; (d) TFA-CH_2Cl_2, 25 °C; (e) RNCO, CH_2Cl_2, 25 °C; (f) NaH, THF, then MeI, 25 °C; (g) triphosgene, CH_2Cl_2, 25 °C then RNH_2; (h) *n*BuLi, THF, -78 °C, then SO_2; (i) N-chlorosuccinimide, CH_2Cl_2, 25 °C.

Additionally, the synthesis of a number of heterocyclic indoline sulfonamides is detailed in Scheme 3. The 1,2,4-oxadiazoles **7** were prepared by condensation of the indoline N-hydroxyguanidine intermediate, while the 4-alkyl thiazole analogs **8** were synthesized by condensation[6] of 5-bromoindoline thiourea with the requisite α-chloroketone. The 4-alkylpyrimidine analogs **9** were obtained by displacement of the appropriate 4-alkyl-2-chloropyrimidines,[7] while the 6- and 5-alkyl-2-pyridine compounds **10** and **12b** were prepared via sequences employing Wittig methodology to introduce the alkyl appendage and chloride displacement to install the indoline moiety. The 3-alkylphenyl analogs **11** were readily prepared from 5-bromoindoline[8] via direct displacement[9] on fluorobenzonitrile, nitrile reduction and a Wittig reaction-hydrogenation sequence to effect conversion to the desired alkyl appendage.



Scheme 3. *Reagents:* (a) BrCN, ether; (b) $H_2NOH{\cdot}HCl$, pyridine, dioxane; (c) RCOCl, pyridine, reflux; (d) $ClSO_3H$, 0 °C; (e) **3**, pyridine, CH_2Cl_2, 25 °C; (f) TFA-CH_2Cl_2, 25 °C; (g) KSCN, aq HCl, reflux; (h) $RCOCH_2Cl$, THF, reflux; (i) *n*BuLi, THF, -78 °C, then SO_2; (j) N-chlorosuccinimide, CH_2Cl_2, 25 °C; (k) 2-chloro-4-alkyl-pyrimidine, Ph_2O, 160 °C; (l) *n*BuLi, THF, 0 °C, then 2,6-dibromopyridine, 25 °C; (m) *n*BuLi, THF, -78 °C, then DMF; (n) $R'CH{=}PPh_3$, THF, -78 °C to 25 °C (R' = *n*Bu, *n*Hex or cyclopentylmethyl); (o) H_2, Pd-C, EtOAc (p) NaH, DMSO, then 3-Fluorobenzonitrile, 25 °C; (q) DIBAL, toluene, -30 °C to 25 °C; (r) *n*BuLi, 0°C, then 2-chloro-5-(1-hexenyl)-pyridine, 25 °C.

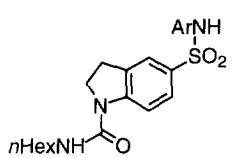
Finally, the 5-octyl oxazole analog **13c** was prepared from commercially available 2-nitrophenethylalcohol as illustrated in Scheme 4. The oxazole ring was formed by aza-Wittig reaction[10] of an appropriately substituted isocyanate, and the closure of the indoline ring was easily accomplished under Mitsunobu conditions.[11]



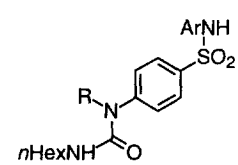
Scheme 4. *Reagents:* (a) AcCl, pyr, CH_2Cl_2, 0 °C; (b) H_2, Pd-C, EtOAc; (c) NBS, DMF, 25 °C; (d) phosgene, CH_2Cl_2, 0 °C, then Et_3N; (e) *n*OctCOCH$_2$N$_3$, PPh_3, THF, 25 °C; (f) LiOH, THF-MeOH-H_2O; (g) DEAD, PPh_3, THF; (h) *n*BuLi, THF, -78 °C, then SO_2; (i) N-chlorosuccinimide, CH_2Cl_2, 25 °C; (j) **3**, pyridine, CH_2Cl_2, 25 °C; (k) TFA-CH_2Cl_2, 25 °C.

Results and Discussion. Compounds were tested in vitro for their ability to stimulate increases in cAMP in CHO cells expressing the cloned human β_3 receptor, as well as in cloned human β_1 and β_2 adrenergic receptors.[12] The results are shown in Table 1, and indicate that many of the N-substituted indoline sulfonamides prepared were potent and selective β_3 agonists. Variation of alkyl substitution often resulted in marked differences in potency, with those compounds bearing octyl sidechains being nearly equipotent to their hexyl-substituted analogs in the heterocyclic series, but three- to tenfold more potent in the urea series. The methyl-substituted analogs were invariably much less active at the β_3 receptor. Furthermore, the N-monosubstituted ureas **5** were more potent than their N-methylated counterparts **6**, which generally showed no advantage over the corresponding N-acyl compounds **4**. Replacement of the urea moiety of **5** with a 5-alkyl-1,2,4-oxadiazole ring (entries 11–13) resulted in a decrease in potency, while the oxazole compound **13c** exhibited somewhat greater potency. Introduction of the 4-alkyl thiazole ring system (entries 14–17) resulted in a dramatic enhancement of the potency and selectivity, with the hexyl (**8b**) and octyl (**8c**) compounds both exhibiting subnanomolar potency at β_3 and excellent selectivity over β_1 and β_2 binding. The bioisosteric 6-alkylpyridine compounds **10** exhibited qualitatively similar potency at the β_3 receptor, but their enhanced affinity for the β_1 and β_2 receptors translates into a significant decrease in selectivity. Replacement of the pyridine with a pyrimidine ring (entries 18 and 19) decreased the β_1 and β_2 affinity while still maintaining good potency at the β_3 receptor, and incorporation of a phenyl ring (entries 23–25) resulted in even greater selectivity relative to their pyridine counterparts.

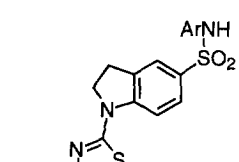
Comparison of the data for compounds possessing the indole-5-sulfonamide moiety with those of their N-unsubstituted and N-methyl substituted aniline counterparts permits a qualitative assessment of the role played by the indoline ring carbons in β_3 agonist activity (entries 28–31). In the urea series, the indoline analog **5b** exhibits potency and selectivity comparable to **1b**, while the N-methyl urea **1c** was markedly less active at the β_3 adrenoceptor. A similar but less dramatic effect is observed in the thiazole series, where **8c** and **14c** are roughly eqipotent while N-methyl compound **15c** is sixfold less potent than indoline thiazole **8c**. These results suggest



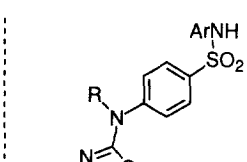
5b



1b R = H
1c R = Me



8c



14c R = H
15c R = Me

that conformational effects involving rotation around the N-C(aryl) bond account for much of the significant differences in β_3 activity and selectivity among the compounds in these series, and that the more favorable conformation for β_3 receptor binding by compounds like **1b** and **14c** is likely that in which the proximal N-H bond is situated nearly coplanar with the aromatic ring. Such a spatial orientation is disfavored by the presence of the N-methyl substituent in **1c** and **15c** but is ensured by the presence of the indoline ring in compounds like **5b** and **8c**, and thus imparts on the N-substituted indoline analogs a degree of β_3 binding affinity which is not observed in their N-methyl counterparts.

Table 1. Comparison of β_3 AR agonist activity and β_1 and β_2 binding affinities[12]

Entry	Compound	β_3 EC_{50}, nM (%act)[a]	β_1 Binding IC_{50},nM[b]	β_2 Binding IC_{50},nM[b]
1	**4b**	88 (60)	10,000	2,000
2	**4c**	7.0 (60)	1,800	1,000
3	**5a**	55 (53)	100,000	7,300
4	**5b**	6.9 (39)	40,000	36,000
5	**5c**	0.73 (56)	1,000	630
6	**6a**	(8)[c]	9,000	7,000
7	**6b**	47 (52)	10,000	6,500
8	**6c**	18 (60)	970	1,000
9	**6d**	55 (57)	10,000	2,500
10	**6e**	(17)[c]	3,000	8,000
11	**7b**	13 (71)	600	52
12	**7c**	31 (59)	580	100
13	**7f**	(20)[c]	330	42
14	**8a**	8.1 (90)	4,000	750
15	**8b**	0.90 (74)	1,400	510
16	**8c**	0.93 (86)	2,600	1,300
17	**8f**	3.2 (90)	4,000	900
18	**9b**	5.1 (81)	1,400	620
19	**9c**	2.4 (60)	1,000	530
20	**10b**	1.2 (78)	900	140
21	**10c**	1.0 (72)	220	63
22	**10f**	1.7 (71)	3,000	450
23	**11b**	1.8 (83)	700	460
24	**11c**	1.3 (69)	2,500	1,500
25	**11f**	5.0 (77)	4,000	7,500
26	**12b**	1.6 (99)	1,800	240
27	**13c**	3.7 (71)	4,200	180
28	**1b**	6.3 (70)	8,000	3,000
29	**1c**	(20)[c]	10,000	10,000
30	**14c**	0.80 (70)	650	340
31	**15c**	5.5 (84)	1500	440

[a]Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol; EC_{50} values are reported 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] Single point data, % activation at 100 nM.

Conclusion. The structure–activity relationship within a series of β3 adrenergic receptor agonists possessing an N-substituted indoline-5-sulfonamide pharmacophore has been investigated. Incorporation of a wide range of appropriately substituted urea, aromatic or heterocyclic moieties on the indoline nitrogen results in a number of exceptionally potent and selective human β3 adrenoceptor agonists. In vivo evaluation of these compounds for their ability to increase metabolic rate is in progress and will be reported in due course.

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