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Identification and SAR around *N*-{2-[4-(2,3-dihydro-benzo[1,4]dioxin-2-ylmethyl)-[1,4]diazepan-1-yl]-ethyl}-2-phenoxy-nicotinamide, a selective α_{2C} adrenergic receptor antagonist

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ABSTRACT

The discovery of the CNS-penetrant and selective α_{2C} adrenergic receptor antagonist *N*-{2-[4-(2,3-dihydro-benzo[1,4]dioxin-2-ylmethyl)-[1,4]diazepan-1-yl]-ethyl}-2-phenoxy-nicotinamide, 13 is described. Structure–activity studies demonstrate the structural requirements for binding affinity, functional activity, and selectivity over other α_2-AR subtypes.

The α_{2C}-AR is a G-protein Coupled Receptor (GPCR) that belongs to the Adrenergic Receptor (AR) subfamily, which contains nine AR subtypes grouped into α_1, α_2, and β subfamilies. The α_2-ARs are involved in presynaptic control of neurotransmitter release and modulate norepinephrine (NE) and serotonin (5HT) neurotransmission.[1–3] There is considerable evidence for 5HT and NE dysfunction in depression, as many marketed antidepressants are known to elevate these neurotransmitters.[4] There are three α_2-AR subtypes: α_{2A}-AR, α_{2B}-AR, and α_{2C}-AR; α_{2A}-AR is expressed in brain and the periphery, α_{2B}-AR is localized in the periphery, while α_{2C}-ARs are highly localized in specific brain regions associated with mood disorders. Data from α_{2C}-AR genetically modified (α_{2C}-AR−/−) mice, though inconclusive, suggest that α_{2C}-AR selective antagonists could have therapeutic utility in treating depression without inducing some of the side effects generally linked with non-subfamily selective α_2-AR inhibitors.[5] Furthermore, there have been reports of selective α_{2C}-AR antagonists that are active in in vivo animal antidepressant models.[6–10] We therefore set out to identify novel α_{2C}-AR selective antagonists that are CNS-penetrant, selective for α_{2C}-AR over α_{2A}-AR, and α_{2B}-AR, and selective over other known CNS neurotransmitter targets. Through optimization of HTS hit **1**, we discovered compound **13** which meets all of our criteria.

A high-throughput screen of our internal compound file utilizing an in vitro competitive radioligand binding assay for α_{2C}-AR was run, using cell membranes prepared from CHO cells overexpressing recombinant human α_{2C}-AR. Hits were then screened in a similar assay format against α_{2A}-AR and α_{2B}-AR. Potent compounds with selectivity for α_{2C}-AR were then evaluated in whole cell functional assays utilizing cell lines overexpressing the α_2-AR isoforms to measure the inhibition of AR (NE) induced calcium mobilization using a FLIPR (fluorescent imaging plate reader). Compound **1,** was identified as a promising lead, with good potency and 15-fold selectivity over α_{2A}-AR and 5-fold selectivity over α_{2B}-AR in the binding assays, but marginal selectivity in the functional assays, being equipotent between α_{2B}-AR and α_{2C}-AR, and only 7× selective over α_{2A}-AR (Table 1).

In order to probe the chemical space around compound **1**, we designed a virtual library of 1134 analogs as depicted in Figure 1. The 1,4-benzodioxan structural feature of compound **1** has been previously incorporated into competitive α-AR antagonists,[11] and this moiety was kept constant in the library design. The central diamine moiety was varied with the first diversity element comprised of 9 cyclic or bicyclic examples with varying lengths and presentation angles, and a second diversity element comprised of 126 alky, heteroalkyl, aryl, and heteroaryl containing building

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Table 1
Compound structure, binding affinities, and functional data illustrating the development of compound **13** and SAR for the series

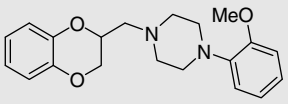
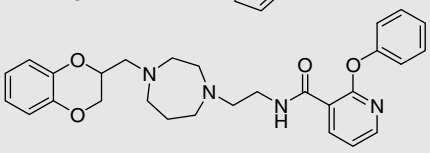
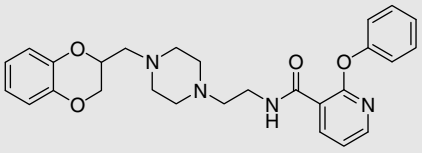
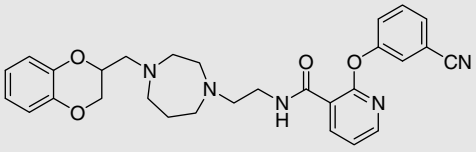
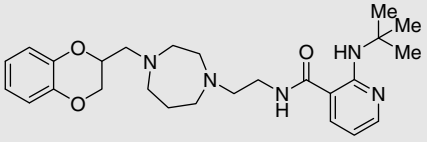
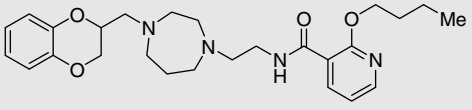
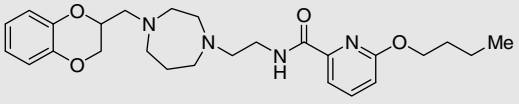
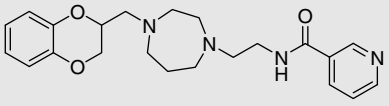
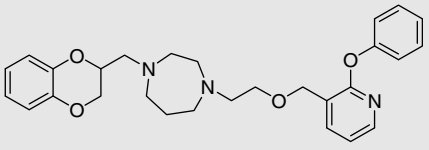
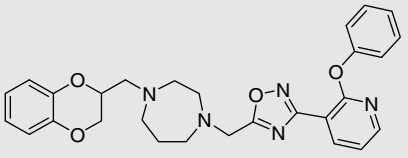
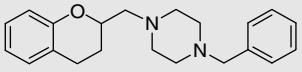
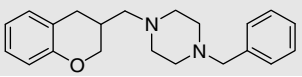
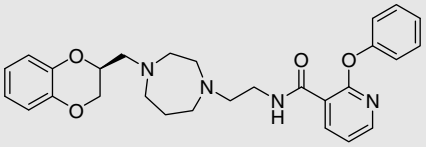
Compound	Structure	Binding K_i (nM)			Binding sel		Functional K_i (nM)			Functional sel	
		α_{2C}	α_{2B}	α_{2A}	α_{2B}/α_{2C}	α_{2A}/α_{2C}	α_{2C}	α_{2B}	α_{2A}	α_{2B}/α_{2C}	α_{2A}/α_{2C}
1		33	194	506	6	15	23	17	167	1	7
2		5	49	220	10	44	25	132	474	5	19
3		3	8	27	3	9	NT	NT	NT		
4		0.8	36	208	48	275	1.9	27.5	501	14	262
5		0.5	28	138	58	286	30.5	103	366	3	12
6		0.8	10.5	81	13	103	4.8	13.3	73.6	3	15
7		0.6	10.9	118	18	195	3.8	14.1	48.4	4	13
8		15.7	17.9	443	1	28	NT	NT	NT		
9		2.5	69.5	66.1	28	26	6.8	132	275	19	41
10		1.9	15.3	44.8	8	23	1.7	5.2	15.4	3	9
11		19.6	72.8	146.1	4	7	21.1	180	167	9	8
12		>500	>500	>500			NT	NT	NT		

Table 1 (*continued*)

Compound	Structure	Binding K_i (nM)			Binding sel		Functional K_i (nM)			Functional sel	
		α_{2C}	α_{2B}	α_{2A}	α_{2B}/α_{2C}	α_{2A}/α_{2C}	α_{2C}	α_{2B}	α_{2A}	α_{2B}/α_{2C}	α_{2A}/α_{2C}
13		0.9	14.3	94.4	16	106	1.9	31.3	>442	16	>232

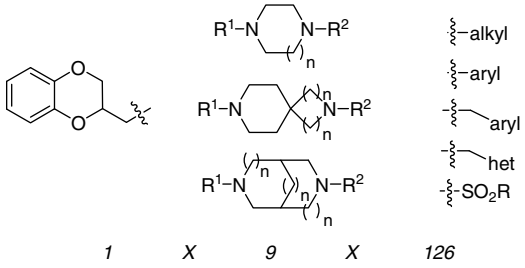
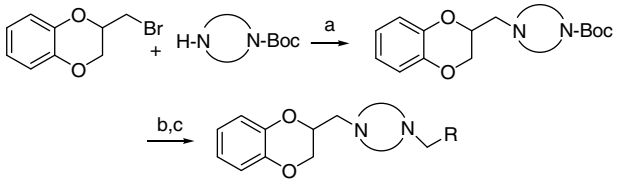


Figure 1. Library design for 'cherry-picked' synthesis.

blocks. We picked approximately 500 compounds for synthesis based on the adherence of their calculated properties to the 'rule of 5',[12] the constraints of the polar surface area that would hypothetically allow for the compounds to cross the blood–brain barrier (BBB),[13] and structural diversity. The compound library synthesized was a 'cherry-picked' selection[14] based on desired calculated properties, rather than a traditional combinatorial cross. Compounds were synthesized on a Tecan liquid handler following the procedure outlined in Scheme 1.



Scheme 1. General synthetic route for library synthesis (compounds **2** and **3**, Table 1). Reagents and conditions: (a) DIPEA, TEA, DMF, 70 °C, 18 h; (b) HCl in dioxane, methanol, rt, 4 h; (c) RCH_2Br, *i*-Pr_2EtN, DMF, 70 °C, 18 h, 5–90% overall.

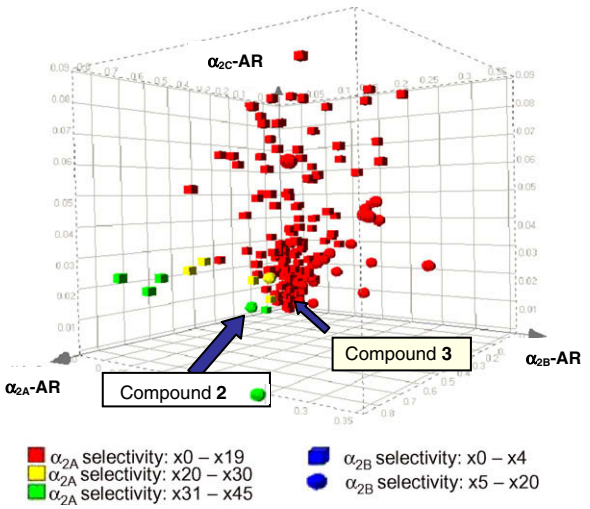


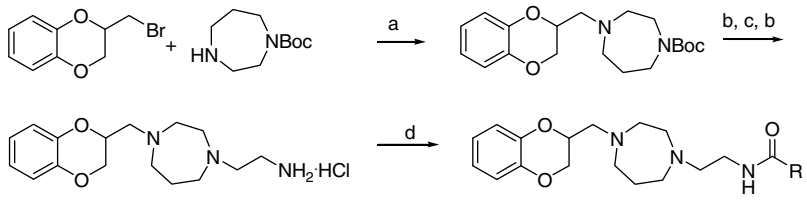
Figure 2. Representation of the 'cherry-picked' library in 3D 'selectivity' space, with axes depicting binding affinity in each of the α_2-AR subtypes. Best compounds are green spheres near the bottom of the visualization.

The compounds were tested in the α_{2C}-AR, α_{2B}-AR, and α_{2A}-AR binding assays, and the results are depicted in a three dimensional α_2-AR activity matrix in Figure 2. Selectivities of the compounds for α_{2C}-AR over α_{2A}-AR are highlighted by color (green is optimal, >30-fold), and the selectivities of the compounds for α_{2C}-AR over α_{2B}-AR are highlighted by shape (spheres are optimal, >5-fold). From this first round 'cherry-picked' library only compound **2**, with 44-fold selectivity over α_{2A}-AR and 10-fold selectivity over α_{2B}-AR (Table 1), emerges as a viable lead. The binding potency and selectivity of **2** are mirrored in the functional subtype data. The remainder of the library contains compounds with potent binding affinities to α_{2C}-AR, but with poor selectivity profiles. One such example is compound **3**, the piperazine analog of **2**, with 9-fold selectivity over α_{2A}-AR and 3-fold selectivity over α_{2B}-AR; this highlights the importance of the central homopiperazine to the selectivity of compound **2** (Table 1).

We then made a series of analogs probing the SAR around the amide portion of compound **2**. Scheme 2 outlines the general procedure used to vary the amide; commercially unavailable carboxylic acids were synthesized according to the literature precedent,[15] but adapted to the microwave which reduces side products and increases the yield.

The binding potencies and selectivity profiles for the analogs in this targeted set are depicted in Figure 3. The visualization shows that compound **2** represents a vein of subtype selective chemical space rather than an isolated selective compound. Optimally selective compounds (>50-fold selective for α_{2C}-AR over α_{2A}-AR and >10-fold selective for α_{2C}-AR over α_{2B}-AR) are again represented by green spheres. Examination of the plot reveals many green spheres, indicating improved selectivity distribution of this targeted set of compounds.

Table 1 shows a sampling of compounds (**4**–**8**) that illustrate the SAR of the series. Compound **4** is very potent and selective in both binding and functional assays (>250-fold selectivities over α_{2A}-AR for both binding and functional activities). This illustrates that *meta*-cyano substitution of the phenyl ring of the nicotinic acid is beneficial for both potency and selectivity in binding and functional determinations. However, we found that all of the compounds with alkylamino or alkoxy substituents instead of the phenoxy have excellent binding potencies and selectivities, but poor functional selectivities. For example, the *tert*-butyl amine analog (**5**) has high levels of potency and selectivity in the binding assays (58-fold and 286-fold over α_{2B}-AR and α_{2A}-AR, respectively), but low levels of potency and selectivity in the functional setting (3-fold and 12-fold over α_{2B}-AR and α_{2A}-AR, respectively). Compounds **6** and **7** illustrate this effect with an *O*-*n*-butyl moiety either directly replacing the phenoxy in compound **2** or with the alkoxy and pyridyl nitrogen transposed. Complete removal of the phenoxy moiety results in low levels of binding selectivity (**8**, 1-fold and 28-fold over α_{2B}-AR and α_{2A}-AR, respectively).



Scheme 2. General synthetic route to access nicotinic amide variants of **2**, compounds **4**–**8**. Reagents and conditions: (a) DIPEA, TEA, DMF, 70 °C, 18 h, 94%; (b) HCl in dioxane, methanol, rt, 4 h, 95%; (c) *tert*-butyl 2-bromoethylcarbamate, DIPEA, DCE, 60 °C, 18 h, 90%; (d) RCOOH, HBTU, TEA, DMF, rt, 5–30%.

Compounds **9** and **10** are examples of modifications to the spacer between the homopiperazine and the nicotinic acid, and were synthesized according to Scheme 3. In general, we found that the less constrained linkers offer better selectivity, which may be true only because the constrained linkers tested are not in the optimal conformation. For example, replacement of the amide with an ether linkage results in good functional activity and selectivity (7 nM, 19-fold and 41-fold over α_{2B}-AR and α_{2A}-AR, respectively); however, the more conformationally constrained oxadiazole spacer does not show good binding or functional selectivity, although it is very potent in both settings (2 nM). Compounds **11** and **12** contain the regioisomers of chroman as replacements for the benzodioxan. Although they are analogs of the less optimal benzyl piperazine, they illustrate that the aminoethanol moiety in the benzodioxan is crucial for α_2-AR activity. The chroman analog of compound **2** could be an interesting analog to test. Compounds **11** and **12** were synthesized analogously to Scheme 1; the bromomethyl chroman starting material for analog **12** is synthesized according to the literature precedent,[16] and the regioisomer used in compound **11** is commercially available.

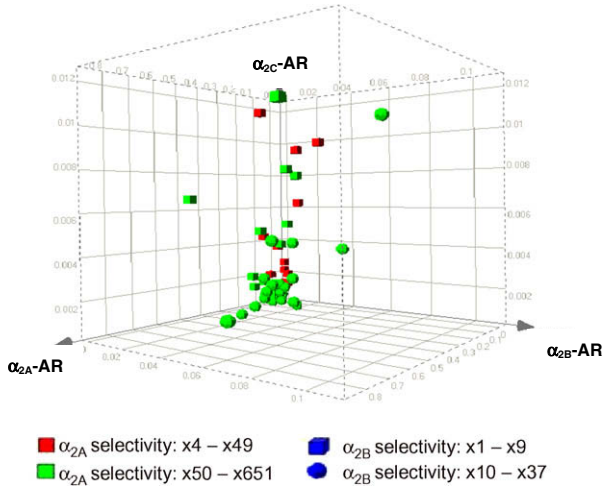
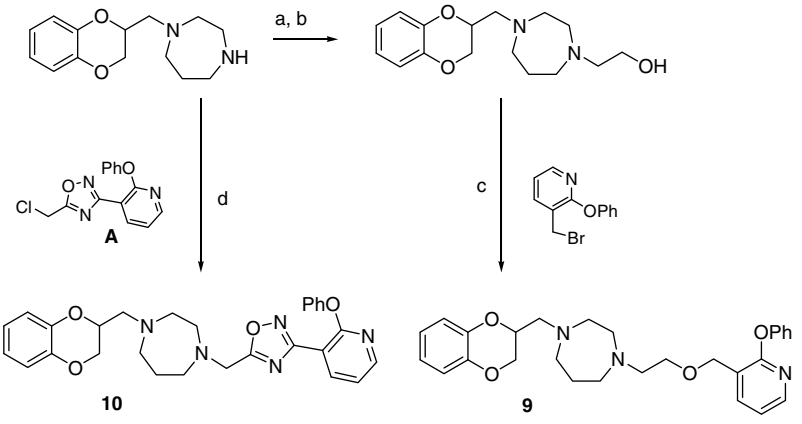


Figure 3. Representation of the second round targeted library in 3D 'selectivity' space, with axes depicting binding affinity in each of the α_2-AR subtypes. Best compounds are green spheres near the bottom of the visualization.

Considering that compounds **1** through **12** are chiral, we chose to resolve compound **2** into its enantiomers in order to study the effect of stereochemistry on potency and selectivity. We found that compound **13**, the S-enantiomer of compound **2** (94% ee), has excellent potency and selectivity in both the binding and functional assays, with functional activity of 2 nM and functional selectivites of >230-fold over α_{2A}-AR and 16-fold over α_{2B}-AR. The R-enantiomer of compound **2** (60% ee, not shown) is less potent than the S-enantiomer (9.2 nM, binding assay), and is less selective, at 12-fold and 53-fold over α_{2B}-AR and α_{2A}-AR, respectively, in the binding assay (functional data not available). The enantiomerically enriched benzodioxans were synthesized according to the literature procedures.[17]

Although compounds **13** and **4** essentially have equivalent potency and selectivity profiles, compound **13** was chosen for further testing. Resolution of compound **4** could provide an extremely potent and selective compound, but we chose to move forward with **13** due to its lower molecular weight and PSA, which are predictive of better brain penetration. Binding affinity of **13** for the rat ortholog of α_{2C}-AR was determined to be 1.7 nM, using an in vitro competitive radioligand binding assay analogous to that applied for the human ortholog. Rat α_{2A}-AR and α_{2B}-AR binding affinities were 177 nM and 7.6 nM, respectively, providing a sufficient window of >100-fold selectivity for α_{2C}-AR over α_{2A}-AR; the 4-fold selectivity over α_{2B}-AR in rat is recognized as low, but is acceptable for



Scheme 3. Synthetic routes to compounds **9** and **10**. Reagents and conditions: (a) ethylchloroacetate, TEA, DMF, 72%; (b) LAH, THF, 56%; (c) NaH, THF, 10% (3-(bromomethyl)-2-phenoxypyridine from NBS bromination of 2-phenoxy-3-pyridinemethanol, 47%); (d) **A**, DIPEA, DMF, 180 °C, 14%; note compound **A** was made analogously to the literature precedent.[18]

Table 2
Compound **13** activities in a panel of human CNS targets

α_{2C}-AR	1 nM	D4	10% at 1 μM
α_{1A}-AR	59 nM	M1	4% at 10 μM
5HT1a	141 nM	M2	1600 nM
5HT1b	44% at 10 μM	M3	27% at 10 μM
5HT2a	2400 nM	NET	1790 nM
5HT2b	690 nM	DAT	3480 nM
5HT7	530 nM	SERT	1480 nM
D2	820 nM	H1	33% at 1 μM
D3	75 nM	H2	1900 nM

Table 3
Drug exposure of **13** in plasma, brain and cerebrospinal fluid (CSF) of Male Wistar–Kyoto rats following subcutaneous administration

Dose (mg/kg)	Plasma (ng/ml)	Brain (ng eq/q)	CSF (ng/ml)	CSF/α_{2C} K_i
3	744	173	7.39	9
10	2100	630	26.9	33
30	6190	2230	113	137

looking at CNS effects in rats, since α_{2B}-AR is only expressed in the periphery. We also tested compound **13** in an assay panel of other CNS targets (Table 2) and found that it is >50-fold selective over α_{1A}-AR and D3, and >100-fold selective over all other targets tested, again supporting that the compound can be used to identify CNS effects of α_{2C}-AR modulation. There is some concern about the relatively high molecular weight and polar surface area of compound **13** (488.6 and 76.2, respectively), which could limit the CNS exposure of the compound. However, subcutaneous drug dosing of compound **13** in male Wistar–Kyoto rats showed adequate drug exposure in the cerebrospinal fluid (CSF). Table 3 shows the exposure of the compound in the plasma, brain, and CSF; the ratio of compound exposure in the CSF versus the α_{2C}-AR K_i's indicates that adequate exposure should be achieved for observation of CNS activity.

In conclusion, through high-throughput screening, 'sparse matrix' library synthesis and traditional medicinal chemistry, we have identified in compound **13** a useful chemical tool for exploring the role of α_{2C}-AR in rat CNS models. Compound **13** has excellent binding affinity and functional activity for α_{2C}-AR, outstanding selectivity among CNS-related targets, excellent binding affinity and selectivity in rats, and adequate exposure in the rat CSF and brain.

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