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Combinatorial Synthesis of 3-(Amidoalkyl) and 3-(Aminoalkyl)-2-arylindole Derivatives: Discovery of Potent Ligands for a Variety of G-protein Coupled Receptors

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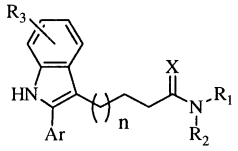
Abstract—Preparation and screening of mixture libraries based on a 2-arylindole scaffold resulted in the discovery of potent ligands for a variety of G-protein coupled receptors.

Combinatorial chemistry has emerged as a powerful tool for the discovery and optimization of new leads in the pharmaceutical industry.[1,2] Previously we have reported the preparation of libraries resulting in the discovery of potent and selective receptor antagonists,[3] as well as various protease inhibitors.[4] In recent years, however, the field of combinatorial chemistry has moved from the preparation and screening of mixture libraries to that of single, purified compounds. This has been largely due to advances in high-throughput screening as well as more efficient parallel synthesis and purification techniques. In this paper we report our findings on the preparation and screening of a series of 2-aryl indole libraries. The results demonstrate that combinatorial mixture synthesis and screening can still play a vital role in the discovery of new leads for a variety of targets.

The key advantage of mixture synthesis lies in the ability to prepare many analogues in a single synthetic operation. Because small changes in a molecular structure can often result in dramatic activity differences the preparation of large numbers of compounds via mixture technology becomes an attractive option. Thus we chose to prepare a series of mixture libraries targeted at several G-protein coupled receptor targets.

The basis for our selection of the 2-aryl indole scaffold comes from the fact that indole derivatives are ubiquitous as an element in many biologically active compounds. We hoped to expand on our previous experience with indole synthesis,[5] taking advantage of the relative ease of substitution of both the indole ring itself as well as the aromatic substituent in the 2-position. We reasoned that by tethering a basic amine to the 3-position of the indole ring while making small modifications in the 4-, 5-, 6- and 7-positions and placing a variety of aryl substitutions in the 2-position (see Fig. 1)



X = 2H,O

Figure 1. 2-Aryl-3-(amino/amidoalkyl)indole derivatives.

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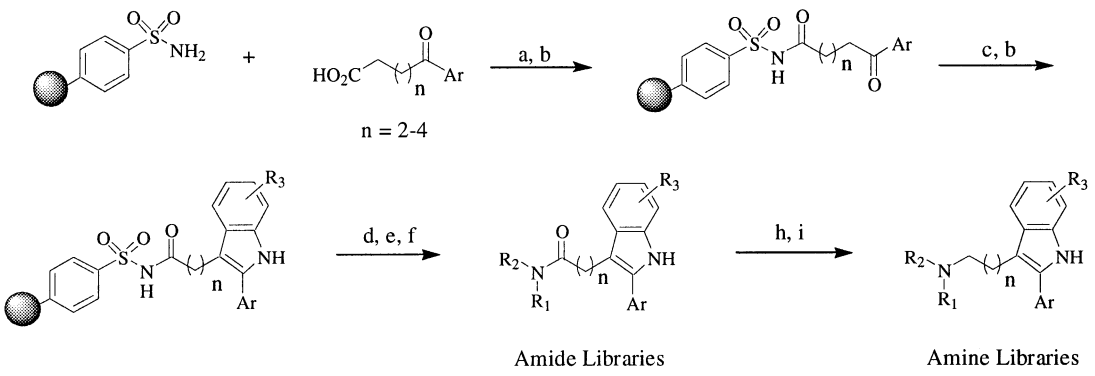
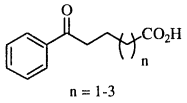
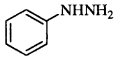
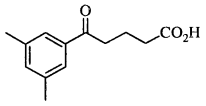
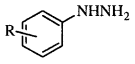
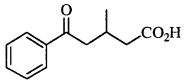
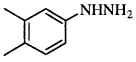
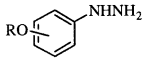
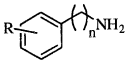


Scheme 1. Solid-phase synthesis 3-(aminoalkyl)-2-arylindoles: (a) DIC, THF/DCM then add resin and DMAP; (b) archive, mix and split resin; (c) aryl hydrazine, $ZnCl_2$, AcOH; (d) pentafluorobenzyl alcohol, PPh_3, DIAD, THF; (e) R_1R_2NH; (f) polystyryl isothiocyanate resin; (g) BMS, dioxane, 50 °C; (h) HCl/MeOH, 50 °C then azeotrop 3×.

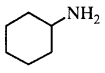
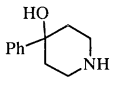
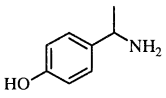
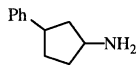
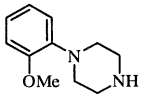
Table 1. Representative library subunits

X Subunits keto-acids	Y Subunits aryl hydrazines	Z Subunits amines
 n = 1-3		
	 o,m,p	
		
	 o,m,p	
		

it would be possible to discover potent and selective compounds for many receptor targets. In order to incorporate an aminoalkyl group into the 3 position of the indole ring we adapted our previously described solid-phase Fischer-indole synthesis[5] to the Kenner safety catch resin.[6] This linker had been shown by Ellman[7] to allow for the use of amine nucleophiles to displace a resin anchored acid and liberate the corresponding amide derivative. Our synthesis is shown in Scheme 1.

Anchoring of the requisite arylalkyl keto acids to the sulfonamide resin was accomplished by preforming the symmetrical anhydride with diisopropyl carbodiimide in tetrahydrofuran/methylene chloride. Subsequent addition of the polymer bound sulfonamide then DMAP and mixing for 20 h gave the desired coupled product. We used 20 different keto-acids as 'X subunits' in this step. After archiving a portion of resin derived from each X subunit for later deconvolution we mixed the pools together and split them into 20 equal portions. The indole cyclization was then carried out with the requisite arylhydrazine in the presence of zinc chloride

Table 2. Assay results for selected Z pools (400 compds/pool), data is reported as % inhibition of ligand binding

	Z1	Z2	Z3	Z4	Z5	Z6	Z7
Assay (concn, μM)							
CCR1 (20)	14	27	7	—	30	57	0
CCR3 (8)	32	52	26	14	10	73	0
CCR5 (8)	1	21	4	10	0	**62**	0
CXCR4 (2)	28	41	26	—	31	33	—
NK_1 (1)	7	23	17	—	2	42	**92**
NK_2 (1)	0	0	10	—	0	0	21
NK_3 (1)	15	9	8	—	20	0	0
5-HT_{1a} (1)	53	21	70	—	30	46	0
5-HT_{2a} (0.1)	81	14	**82**	54	45	63	0
5-HT_{2c} (0.1)	66	21	66	12	0	3	0
5-HT_{5a} (5)	50	6	34	—	23	20	52
5-HT_6 (5)	**97**	76	95	44	87	68	42
NPY_1 (2)	12	8	12	8	24	17	0
NPY_5 (2)	82	89	85	—	**98**	96	23
GnRH (1)	4	7	4	**66**	6	6	—
MCR-4 (2)	10	**62**	17	—	5	23	—

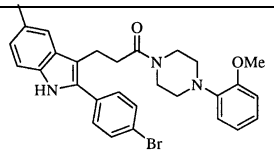
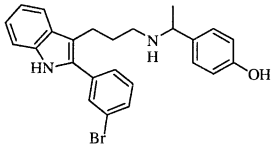
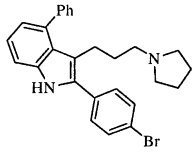
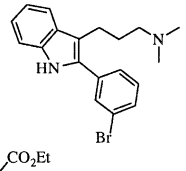
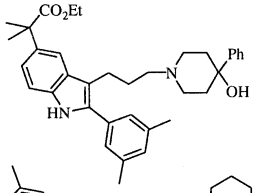
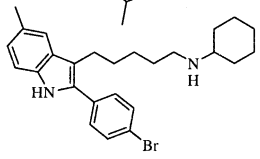
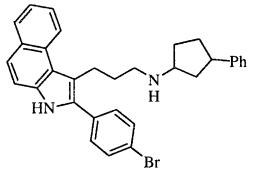
and acetic acid.[5] In this position we chose 20 different hydrazine derivatives (Y subunits). After archiving a portion of each pool (now a mixture of 20 compounds with a known aryl hydrazine) the remaining resin was mixed and split into 80 equal portions. The sulfonamide was alkylated using pentafluorobenzyl alcohol under Mitsunobu conditions provide the activated alkyl sulfonamide. Displacement with 80 amine 'Z subunits' afforded 80 pools of compounds[8] with 400 compounds/pool. We then split the pools in half and treated one half with borane–methylsulfide complex at 50 °C for 3 h. Concentration followed by treatment with methanolic HCl[9] afforded the desired product pools. This gave two libraries; one of amide derivatives and one of amine derivatives each with the same core structures. Each library consisted of 80 pools with 400 compounds/pool for a total of 64,000 compounds. A second pair of libraries was prepared using different X, Y, and Z subunits. In all 128,000 compounds were prepared as 320 pools of 400 compounds. A sample of representative subunits is given in Table 1. As shown keto acids of varying tether length and substitution were used in the 'X' position. In the 'Y' position various hydrazines bearing *ortho*, *meta* and *para* substituents ranging from electron withdrawing to electron donating were used. For the 'Z' subunits, a broad variety of cyclic and acyclic secondary amines such as dialkyl amines as well as piperazine, piperidine and pyrrolidine derivatives were used. Also, aryl alkyl amines and other amines based on privileged structures were incorporated.

The resulting libraries were screened in a wide variety of G-protein coupled receptor-binding assays. Activity was observed in many families of receptors including neurokinin, chemokine and serotonin receptors. A selection of data from this screening effort is shown in Table 2. In the chemokine family we were most interested in the CCR5 receptor due to its recently discovered role in HIV-1 cell entry.[10] Pool **Z6** had a 62% inhibition of MIP-1α binding at 8 micromolar and was followed up by deconvolution. In the serotonin family several pools with activity were observed. For example, pool **Z3** had activity against the 5-HT_{2a} receptor and showed some selectivity versus many of the other receptors in this family. Similarly pool **Z1** showed activity against 5-HT_6. These pools were selected for deconvolution. In the neurokinin family pool **Z7** stood out as both active versus hNK_1 and selective versus all other targets tested. Again this pool was deconvoluted. As can be seen activity versus NPY_5, GnRH and MCR-4 receptors was also observed (pools **Z6**, **Z4**, and **Z2**, respectively).

Z pools of interest were deconvoluted in two stages by resynthesizing the compounds from the archived resin pools (see above). When these studies were complete (data not shown) a series of individual compounds was prepared and screened for activity. This data is shown in Table 3.

Of interest to us, as well as our colleagues working in the substance P area, was compound **1**. Remarkably, **1** was found to have a 0.8 nM affinity for hNK_1 while having no activity for the other receptors in this family. This compound became the starting point for a medicinal chemistry effort to develop a novel selective NK_1 antagonist.[11] For the NPY_5 receptor a potent and selective compound, **2**, was also discovered. Compound **2** has 0.8 nM affinity for NPY_5 while having no activity versus NPY_1. Not surprisingly some compounds were found to have activity in the serotonin family of receptors. For example, compound **3** has 10 nM binding affinity for 5-HT_{2a} and modest selectivity over 5-HT_{2c} and 5-HT_6 (60 and 635 nM, respectively). No activity below 1 micromolar versus the other serotonin receptors is observed for compound **3**. Likewise compound **4** is a potent ligand (0.7 nM) for the 5-HT_6 receptor while possessing good selectivity versus 5-HT_{1a}, 5-HT_7 and 5-HT_{5a}. Compound **4** did however, have activity in 5-HT_{2c} and 5-HT_{2a} (data not shown). Compounds **3** and **4** could be useful leads to help identify potent and

Table 3. Activity of single compounds

Compound	Structure	Activity
1		NK1, 0.8 nM NK_2, > 10 μM NK_3, > 10 μM
2		NPY_5, 0.8 nM NPY_1, > 10 μM
3		5-HT_{2a},10 nM 5-HT_{2c}, 60 nM 5-HT_6, 635 nM 5-HT_{1a}, > 1 μM 5-HT_{5a}, > 1 μM 5-HT_7, > 1 μM
4		5-HT_6, 0.7 nM 5-HT_7, 300 nM 5-HT_{1a}, > 1 μM 5-HT_{5a}, > 1 μM
5		GnRH, 52 nM
6		MCR-4, 612 nM
7		CCR5, 1190 nM CCR1, > 10μM CCR2, > 10μM CCR3, 920 nM CXCR3, > 10 μM CXCR4, 1520 nM

selective ligands for many serotonin receptor family members.

Compound **5** is a potent 52 nM ligand for the GnRH receptor and was of interest to our colleagues on the medicinal chemistry team due to some of its novel structural features. Finally, moderately potent compounds **6** and **7** were discovered as structurally diverse leads for the MCR-4 and CCR5 receptors, respectively.

In summary, we have shown that mixture libraries are a valuable tool in the discovery of new leads for drug discovery projects. Specifically, preparation and screening of mixtures of 2-arylindole derivatives has lead to the discovery of many potent compounds across a variety of receptor targets. Despite the drawbacks of making and screening mixtures of compounds this study exemplifies the power of combinatorial mixture techniques in finding compounds with often unexpected activity and/or selectivity.

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