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PARALLEL SYNTHESIS OF 3-ARYLOXY-2-PROPANOLAMINES AND EVALUATION AS DUAL AFFINITY 5-HT$_{1A}$ AND 5-HT RE-UPTAKE LIGANDS

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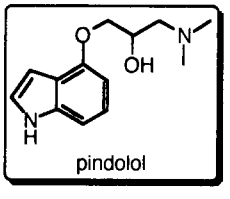
Abstract: A solution phase synthesis for the preparation of 3-aryloxy-2-propanolamine libraries has been developed. This resulted in the identification of **5** as a ligand with dual affinity for 5-HT$_{1A}$ and serotonin re-uptake receptors which shows excellent pharmacokinetic parameters.

Depressive illness constitutes a considerable economic and social cost to society[1]. A number of classes of drugs (*e.g.* MAOI's, TCA's and SSRI's) are currently being used in clinical practice but all have associated unpleasant side effects, a slow onset of action and a large patient population who do not respond to treatment[2]. Microdialysis techniques have shown that the majority of antidepressants act at the biochemical level to elevate levels of synaptic serotonin[3]. However, elevation of serotonin is also associated with negative feedback at presynaptic 5-HT$_{1A}$ autoreceptors which reduces the rate of neuron firing and hence the synaptic concentration of serotonin, until a time dependant down regulation of these receptors occurs.



pindolol

Recent clinical trials have used combination therapies with SSRI's to test the hypothesis that an improvement in response time would be observed if pindolol, a partial agonist at the 5-HT$_{1A}$ receptor, is co-administered to block these autoreceptors[4]. Results from the trials suggested that a greater proportion of patients responded to combination therapies compared to those receiving only SSRI's, and there was some evidence to suggest that reduced treatment time was needed to obtain a sustained response. We were intrigued with the possibility of developing ligands which have dual activity as presynaptic 5-HT$_{1A}$ receptor antagonists and serotonin re-uptake blockers. Such an approach may have pharmacokinetic advantages over combination therapies although obtaining dual ligands with suitable binding profiles is a complex undertaking.

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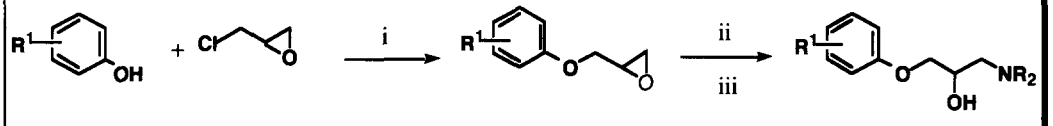
Our approach aimed to modify pindolol by incorporating amine fragments from serotonin re-uptake ligands whilst also using substituted phenols from 5-HT_{1A} ligands to explore SAR. The common occurrence of 3-aryloxy-2-propanolamines as drugs suggested to us that synthesis of libraries based on this template would be a profitable area of exploration which would enable cross-screening at a number of biological targets. A combinatorial approach would offer the advantage that both the amine and phenol substitution could be changed to efficiently probe 3-aryloxy-2-propanolamines for novel leads across a number of therapeutic areas. A solution phase approach[5] was selected since this could be rapidly set up after optimal conditions had been established. Recent publications outline a similar approach towards the preparation of novel β-amino alcohols[6] and silent 5-HT_{1A} antagonists[7].

Epichlorohydrin[8] was an attractive highly functionalised, chiral building block for the preparation of 3-aryloxy-2-propanolamines. A variety of conditions for the opening of epichlorohydrin with phenols or amines were investigated during evaluation of this approach for solution phase chemistry. The optimized conditions for successful reaction with a range of phenols and amines are shown in Scheme 1[9].

Scheme 1

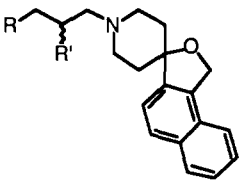


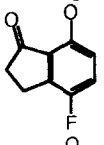
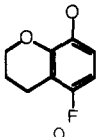
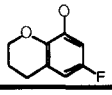
Reagents: i) K_2CO_3, 50°C, 18h; ii) CH_3CN, R_2NH (0.8eq), 50°C, 18h; iii) Amberlyst 15.

Phenols were reacted with epichlorohydrin and potassium carbonate in the absence of solvent. Simple work up gave 1,2-epoxy-3-aryloxypropane together with some 3-aryloxy-1-chloro-propan-2-ol which could be archived. The availability of these reactive advanced intermediates facilitated the preparation of large numbers of compounds for a number of potential therapeutic areas. Addition of amines to the 1,2-epoxy-3-aryloxypropanes and heating in acetonitrile gave access to 3-aryloxy-2-propanolamines which were purified using Amberlyst 15 resin. This methodology was used to synthesize large numbers of compounds aimed at a range of therapeutic areas. Amines and phenols were identified from a set of structurally diverse[10] commercially available reagents and 'privileged' structures[11]. Additionally, we incorporated fragments which are reported to have affinity for 5-HT_{1A}[12] or 5-HT re-uptake[13] receptors in our efforts to modify pindolol in an efficient manner.

Binding data were obtained on purified mixtures containing upto 100 compounds in 100 wells. Wells which showed desired biological activity were first deconvoluted to mixtures of 10 and finally single compounds using this solution phase approach. Compounds of interest were resynthesized and purified using standard chromatography techniques[14]. Whilst we used only simple, commercially available apparatus to carry out this process in parallel, the methodology was amenable to the synthesis of milli or multi-gram quantities of material and we anticipate that robotic techniques could also be used.

Table 1[14]. 5-HT_{1A} and 5HT re-uptake Binding Affinities[a]



Compound	R	R'	5-HT_{1A} (Ki/nM)[b]	5-HT_{1A}[c]		5-HT re-uptake (Ki/nM)[e]
				EC_{50} (nM)	% (5-HT)[d]	
pindolol			24 (21, 28)	27	36	>7000
1	PhO-	-OH (*S*)	1.1	1.2	81	50
2[f]	2-F-PhO-	-OH (*S*)	1.0 (0.7, 1.3)	1.1	100	52
3[g]	2-F-PhO-	-OH (*R*)	6.0 (5.2, 6.9)	21	92	120 (98, 140)
4	3-Et-PhO-	-OH (*S*)	1.7 (1.4, 2.0)	3.3	61	87
5[h]	$PhCH_2$-	-OH	8.3 (7.2, 9.5)	37	87	10 (10, 11)
6	$PhCH_2O$-	-OH	2.2 (1.6, 3.1)	31 (25, 38)	108 +/- 5.5	83 (76, 91)
7[h]	$PhCH_2$-	-H	72	>10,000	-	28 (25, 32)
8		-OH	2.3	3.5	65	45 (39, 51)
9		-OH	2.8	>10,000	-	49
10		-OH	5.5	Inverse	-	72

[a]Data shown are the mean of 2-5 independent determinations with limits of statistical certainty shown in parenthases where n> 2.[b]Displacement of specific [^{3}H] 5-HT binding to cloned human 5-HT_{1A} receptors stably expressed in HeLa cells. [c] Stimulation of [^{35}S] GTPγS binding to cloned human 5-HT_{1A} receptors stably expressed in HeLa cells[15]. [d]Efficacy normalised to the maximal 5-HT response. [e]Displacement of specific [^{3}H] citalopram binding to 5-HT uptake site in rat whole cortex[16]. [f] ee= 92.8% (chiral OD-H 250x 4.6mm, 5% EtOH in hexane, 1mL/min, UV = 210nM); [g] ee= 93% under above conditions. [h] Prepared from ring opening of corresponding epoxides with amine under under the reported conditions.

Phenols bearing simple ethyl and fluoro substituents were identified as a good replacement for the indole moiety of pindolol and showed improved binding affinity at 5-HT_{1A} and serotonin re-uptake receptors (Table 1). Compounds with the 3-aryloxy-2-propanolamine template, such as propranolol and penbutolol are ligands for adrenoceptors and also 5-HT_{1A} receptors. Modelling and mutagenisis studies have shown that an asparagine 386 residue located in helix VII of the human 5-HT_{1A} receptor is crucial for binding of 3-aryloxy-2-propanolamines[17] although structurally unrelated ligands such as WAY100635[18] and 8-OH-DPAT[19] are not affected because they bind in a different manner. Both oxygen atoms of propranolol were demonstrated to be crucial for the binding, and it was suggested that a direct interaction with Asn386 by hydrogen bonding was occurring. The importance of hydrogen bonding has also been demonstrated at β-adrenoceptors[20]. The *S* enantiomer of propranolol had a higher binding affinity indicating a stereoselective component to the interaction and our observations that the *S* enantiomer **2** had higher affinity than the corresponding *R* enantiomer **3** also support this. Replacement of the aryl ether linker by carbon (**5**) maintained binding affinity at the 5-HT_{1A} receptor. This contrasts with identical changes in propranolol[17] where a substantial loss in affinity was observed, and suggests that the ether atom may act solely as a linker and do not participate in hydrogen bonding in our series. Compound **6** suggests that the receptors may be tolerant to changes in the orientation of the aryl ring. Removal of the hydroxyl in addition to the aryl ether linker (**7**) was detrimental for binding affinity, presumably because no hydrogen bonding to Asn386 could occur. No intrinsic activity was observed for **7** in the functional assay.

Incorporation of fragments from 5-HT_{1A} ligands[12] into this series to give **8**, **9** and **10** maintained binding affinity. Interestingly, **10** exhibited inverse agonism wheras no intrinsic activity was observed for the structurally related **9**. Whilst we identified a range of modifications to the indole template present in pindolol that maintained dual affinity and influenced functional efficacy, the affinity at the serotonin re-uptake receptor was very sensitive to the nature of the amine. Indeed, the spirofused piperidine shown in **1**-**10** was the only amine identified from our library work which consistently gave moderate levels of binding affinity at serotonin re-uptake receptors in addition to 5-HT_{1A} receptors. A balanced profile was observed for **5** although this demonstrated almost full agonism at 5-HT_{1A} receptors. This example of a dual ligand was selected for pharmacokinetic evaluation. Excellent systemic exposure was observed following oral dosing in rat (3mg/kg in 1:1 PEG400:water): F = 65%, $t_{1/2}$ 3.0h. The high LogP 3.9[21] facilitated brain penetration (brain-blood ratio: 3-5 @ 15-120min after 0.5mg/kg i.v.) and suggest that **5** may be a useful research tool to validate our original hypothesis *in vivo*.

Versatile chemistry for the preparation of 3-aryloxypropan-2-olamines has been developed to enable libraries to be synthesized using simple apparatus. Screening has identified a number of novel ligands for CNS receptor targets. We have reported that **5** is a ligand with dual affinity for 5-HT_{1A} and serotonin re-uptake receptors which shows excellent pharmacokinetic parameters. We anticipate that 3-aryloxy-2-propanolamines, such as **5**, will provide useful tools to delineate serotonin receptor biology *in vivo*.

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9. Representative Experimental Conditions: Glass tubes (12mm diameter) were charged with 2mmol substituted phenol, 4mmol potassium carbonate and 6mmol epichlorohydrin[8]. The contents were mixed by vortex before the tubes were flushed with nitrogen, sealed and heated at 50°C in a heating block for 18h. Acetonitrile (3mL) was added and the contents mixed. Solid was removed by filtration of individual tubes through a fritted, disposable syringe. Concentration of the filtrate gave the required epoxides together with the corresponding chloro alcohols. These were stored at –78°C until required. Glass tubes were charged with 64μM epoxide , 49μM amine and acetonitrile (1mL). The tubes were flushed with nitrogen, sealed and heated at 50°C in a heating block for 18h. The tubes were allowed to

cool and methanol (1mL) was added to solubilise the contents which were applied to a 5mL disposable syringe containing 4mL of prepared Amberlyst 15 resin. The contents were allowed to stand for 10 minutes before being collected as waste. The Amberlyst 15 was washed successively with a syringe volume of acetonitrile-water (1:1), acetonitrile (2 times), acetonitrile-NH_4OH (4% solution). These washings were discarded. The washings from acetonitrile-NH_4OH (10% then 20% solutions) were collected in pre-weighed tubes and concentrated prior to analysis by HPLC, m/s and NMR (purities obtained were generally >90% for single compounds prepared by this method). A convenient proceedure was to prepare 10mM solutions of the purified product in d_6-DMSO for binding and NMR studies.

10. The selection of the commercially available phenols was made by searching the Available Chemicals Directory (ACD) for all mono-phenols that did not contain additional reactive functional groups. The list was further reduced by only considering a limited number of UK based suppliers. This new list was split into large and small phenols by the application of a molecular weight <300 cut off and these lists were treated to a dissimilarity search using our in-house 2D descriptors: Kearsley, S.K.; Sallamack, S.; Fluder, E.M.; Andose, J.D.; Mosley, R.T.; Sheridan, R.P., *J. Chem. Inf. Comp. Sci.*, **1996**, *36*, 118. From these lists were picked 70 small and 30 large phenols for use within the rapid analogue synthesis: A similar approach was used to select a set of commercially available amines.
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