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Discovery and optimization of (*R*)-prolinol-derived agonists of the Growth Hormone Secretagogue receptor (GHSR)

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ABSTRACT

The discovery and optimization of a novel series of prolinol-derived GHSR agonists is described. This series emerged from a 11,520-member solid-phase library targeting the GPCR protein superfamily, and the rapid optimization of low micromolar hits into single-digit nanomolar leads can be attributed to the solid-phase synthesis of matrix libraries, which revealed multiple non-additive structure–activity relationships. In addition, the separation of potent diastereomers highlighted the influence of the α-methyl stereochemistry of the phenoxyacetamide sidechain on GHSR activity.

The Growth Hormone Secretagogue receptor (GHSR) has been implicated in a number of important biological functions, including the regulation of growth, metabolism, and food intake.[1] Small molecule agonists of GHSR have been studied in the clinic[2] for the treatment of growth hormone disorders, frailty, and cachexia. GHSR is a member of the G-protein-coupled receptor (GPCR) protein superfamily[3], and as part of an in-house effort to identify novel GHSR agonists[4] we screened a series of GPCR-targeted libraries, including one based on a secondary amine chemotype.[5] This library afforded a number of low micromolar GHSR agonist hits, and the identification and optimization of these hits is described herein.

The secondary amine GPCR-targeted library[5] was synthesized on solid support and comprised three reagent sets: R^1, consisting of cyclic and acyclic secondary amino alcohols; R^2, consisting of primary amines and anilines; and R^3, consisting of carboxylic acids. The overall dimensions of the library were 11 $R^1 \times 31$ $R^2 \times 42$ R^3, which in theory would afford 14,322 discrete products. However, each library sample underwent a rigorous quality control (QC) process: each sample was analyzed by HPLC (with UV and ELS detection) and mass spectrometry. Samples which did not afford ⩾70% purity by HPLC and a mass ion corresponding to the desired product were discarded. Upon completion of the QC process, 11,520 samples were submitted for screening against a variety of GPCR targets and resulted in the identification of a series of prolinol-derived GHSR agonists as described in Table 1.

Given that 11,520 library members were screened in the GHSR assay, the high degree of structural homology for the active samples described in Table 1 was compelling. Indeed, a modest amount of SAR could be elucidated from the HTS data, with the caveat that the original library samples were submitted as crude (but ⩾70%

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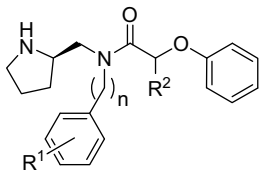
Table 1
Initial hits from secondary amine GPCR library



Entry	R^1	n	GHSR EC_{50}[a,b] (μM)		
			R^2 = H	R^2 = Me	R^2 = Et
1	H	0	>10	3	N.a.
2	*p*-AcNH	0	2	0.3	4
3	*p*-$NCCH_2$	0	N.a.	9	14
4	*m*-MeO	0	>10	4	>10
5	*m*-MeS	0	N.a.	2	>10
6	*o*-MeO-*m*-Ph	0	5	N.a.	2
7	H	1	>10	N.a.	0.9

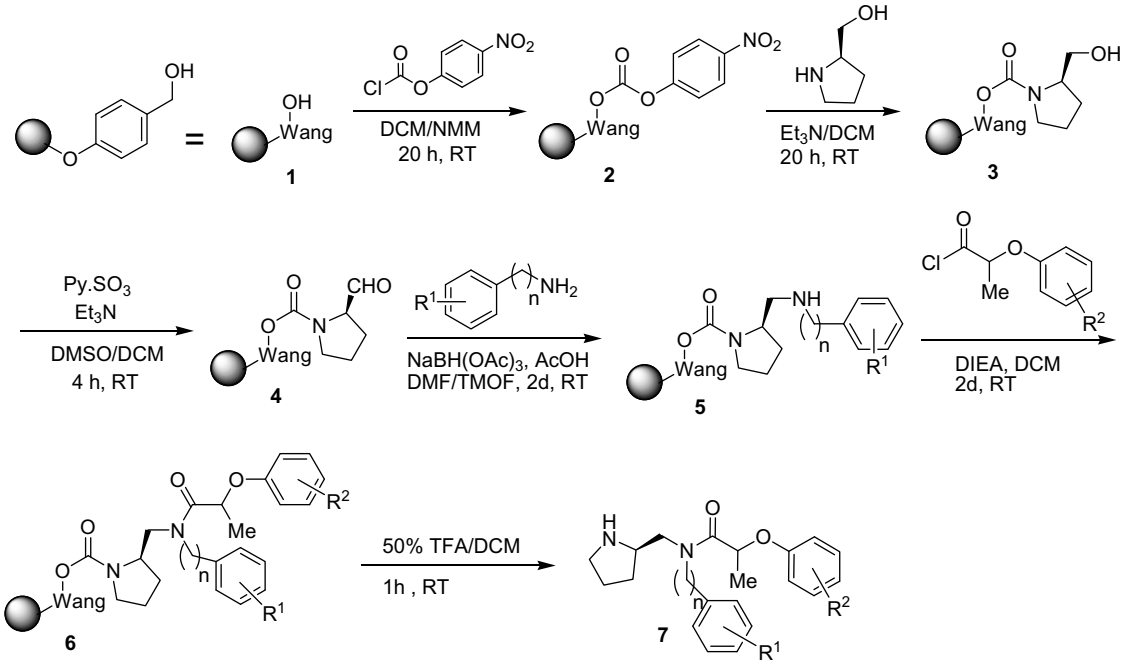
[a] See Ref. 6 for experimental details.
[B] N.a., no sample (failed QC).

pure by HPLC) samples. Moreover, the 31 primary amines (30 anilines and 1 benzylamine) used in the library spanned a wide range of chemical property space and thus the R^1 substituents listed in Entries 1–6 of Table 1 do not constitute a focused library by any stretch of the imagination. The *p*-AcNH substituent (Entry 2) was the most active R^1 substituent in the aniline series ($n = 0$), and it appeared that a methyl group was the optimal R^2 substituent, although the R^2 SAR was confounded by a significant number of missing samples—these samples were not screened because they failed our QC process and were discarded. In addition to the aniline series exemplified by Entries 1–6, a benzylamine analog ($n = 1$) afforded sub-micromolar GHSR agonist activity (Entry 7, R^2 = Et). Because only a single benzylamine reagent was present in the library, the absence of the R^2 = Me sample (which was anticipated to be more potent than the R^2 = Et sample) served to heighten our interest in the benzylamine series.

Analysis of the GHSR screening data for inactive library members indicated that the prolinol-derived secondary amine and α-substituted phenoxyacetamide moieties described in Table 1 were critical for activity. As a result, we initiated the synthesis of follow-up libraries utilizing the solid-phase chemistry (outlined in Scheme 1) which provided the original library[5] Starting with commercially available Wang[7] linker-equipped polystyrene resin **1**, the Wang hydroxyl group was converted to the corresponding *p*-nitrophenyl carbonate **2** under standard conditions[8] followed by *p*-nitrophenol displacement with (*R*)-prolinol to afford solid-supported prolinol carbamate **3**. The primary hydroxyl group of **3** was oxidized to aldehyde **4** using pyridine–sulfur trioxide[9] and the aldehyde subsequently underwent reductive amination with primary anilines ($n = 0$) and benzylamines ($n = 1$) under standard conditions[10] to afford secondary amine **5**. It should be noted that this seemingly innocuous reductive amination step does not proceed smoothly in solution, particularly with anilines. In a deviation from the amide coupling conditions used in the original library (carboxylic acid + coupling reagent), we elected to utilize acid chlorides owing to their enhanced electrophilicity. Thus, treatment of **5** with the appropriate acid chloride and Hunig's base afforded **6**, which was liberated from the solid support via treatment with trifluoroacetic acid to provide the final product **7**. All follow-up samples were purified by reverse-phase HPLC and characterized via LC/MS (⩾95% pure by UV) and ^{1}H NMR.

Through the synthesis of a number of small libraries (containing <20 samples per iteration), we were able to rapidly elucidate the key structure–activity relationships described in Figure 1, including the observations that substitution at the prolinol nitrogen abrogated GHSR activity, *R* stereochemistry was preferred at the 2-position of the pyrrolidine, and an α-methyl group was optimal on the phenoxyacetamide sidechain. As a result, our SAR efforts focused on the two aromatic groups: the aniline (or benzylamine) sidechain and the phenoxy group of the α-methyl 2-phenoxyacetamide sidechain.

At this point, we elected to synthesize a 'matrix' library, in which the aniline/benzylamine and phenoxyacetamide substituents were varied simultaneously. This library was straightforward to synthesize on solid support, as the use of IRORI MicroKans™ and radiofrequency tags facilitated a 'split-mix' synthesis protocol[11] We synthesized a library comprising approximately 40 different



Scheme 1. Solid-phase synthesis of the secondary amine library.