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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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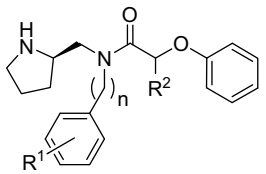
Table 1
Initial hits from secondary amine GPCR library



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

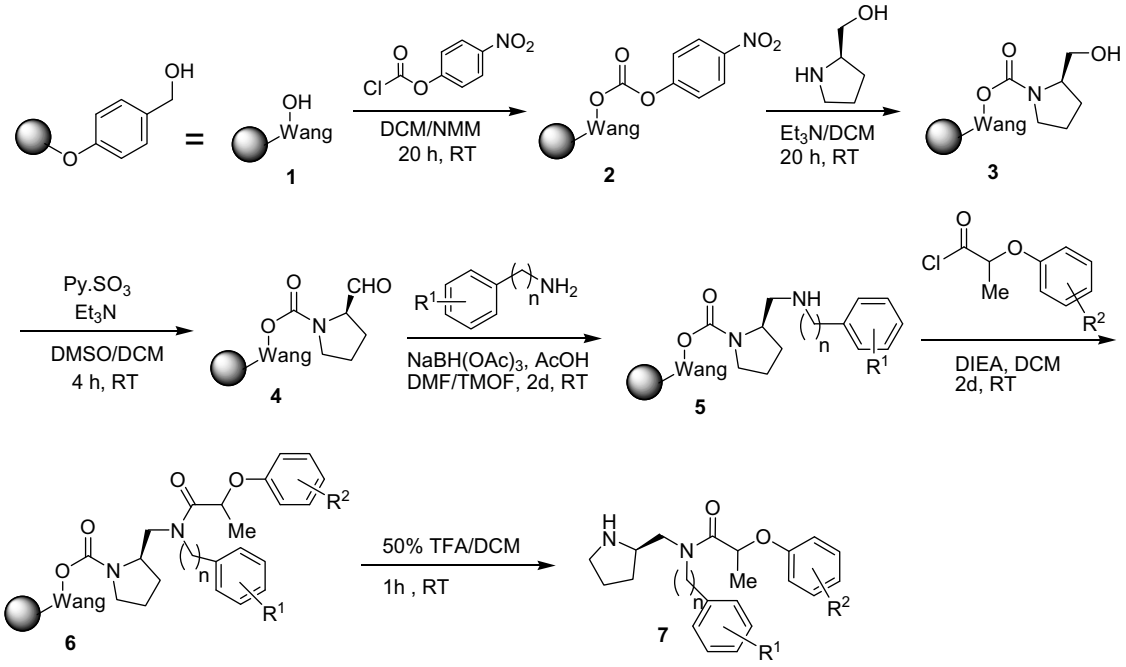
[a] See Ref. 6 for experimental details.
[B] N.a., no sample (failed QC).

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.

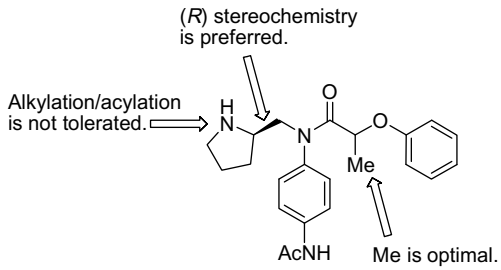
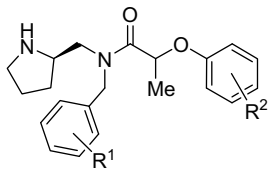


Figure 1. SAR of the secondary amine and phenoxyacetamide sidechains.

Table 2
SAR matrix for the benzylamine series



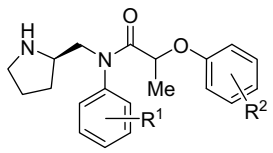
Entry	R^1	GHSR EC_{50} (% Control)[a] (nM)		
		R^2 = H	R^2 = *o*-Cl	R^2 = *m*-Cl
1	H	90 (110)	20 (60)	7 (80)
2	*o*-Me	40 (110)	140 (90)	3 (100)
3	*m*-Me	80 (90)	90 (70)	120 (20)
4	*o*-Cl	150 (100)	260 (60)	40 (80)
5	*m*-Cl	50 (80)	10 (90)	20 (70)
6	*p*-Cl	220 (80)	60 (80)	30 (90)

[a] See Ref. 6 for experimental details.

anilines and benzylamines, and 11 different phenoxyacetamides and screened it for GHSR activity. The most interesting SAR trends are summarized in Table 2 (benzylamine series) and Table 3 (aniline series).

In the case of the benzylamine series (Table 2), it was apparent that the R^1 and R^2 SAR were non-additive; that is, the most potent R^1 substituent when R^2 = H does not continue to be the most potent R^1 substituent when R^2 is varied, and vice-versa. This trend can be clearly seen by comparing Entries 2 and 5: when R^2 = H or R^2 = *m*-Cl, the most potent GHSR agonists were found when R^1 = *o*-Me (Entry 2, EC_{50} = 40 and 3 nM, respectively); when R^2 = *o*-Cl, the most potent GHSR agonist was found where R^1 = *m*-Cl (Entry 5, EC_{50} = 10 nM). It is also interesting to compare Entries 2 (R^1 = *o*-Me) and 3 (R^1 = *m*-Me); in this case, similar potencies were observed when R^2 = H or R^2 = *o*-Cl, but when R^2 = *m*-Cl the SAR diverged dramatically, R^1 = *o*-Me (Entry 2) afforded a 3-nM full agonist, whereas R^1 = *m*-Me (Entry 3) provided a 120-nM weak partial agonist.

Table 3
SAR matrix for the aniline series



Entry	R^1	GHSR EC_{50} (% Control)[a,b] (nM)		
		R^2 = H	R^2 = *o*-Cl	R^2 = *m*-Cl
1	H	440 (80)	I.a.	120 (60)
2	*m*-AcNH	1000 (80)	200 (70)	140 (60)
3	*p*-AcNH	330 (80)	50 (90)	30 (90)
4	*o*-Cl	1260 (80)	I.a.	780 (70)
5	*m*-Cl	90 (110)	250 (60)	40 (110)
6	*p*-Cl	600 (80)	I.a.	120 (90)

[a] See Ref. 6 for experimental details.
[b] I.a., inactive ($EC_{50} \geqslant 10$ μM).

As shown in Table 3, non-additive SAR was also observed in the aniline series. For example, the most potent GHSR agonist when R^2 = *o*-Cl or R^2 = *m*-Cl was Entry 3 (R^1 = *p*-AcNH), affording full agonists with EC_{50}s of 50 and 30 nM, respectively. However, when R^2 = H, the most potent GHSR agonist was Entry 5 (R^1 = *m*-Cl). It is also noteworthy that even though a significant loss of activity was observed when R^2 = *o*-Cl—three analogs (Entries 1, 4, and 6) were inactive and two others (Entries 2 and 5) were partial agonists—Entry 3 (R^1 = *p*-AcNH) was one of the most potent compounds in the entire matrix.

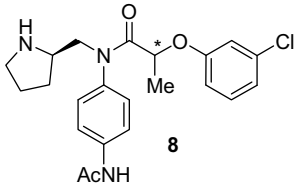
Although the ramifications of non-additive SAR are context-dependent, the knowledge that perturbations in one region of a chemical series are not synergistic with perturbations in another region of the same chemical series is clearly pertinent to any medicinal chemistry effort. In our experience, non-additive SAR is often observed with GPCR targets, possibly due to the inherent mobility of the membrane-spanning α-helices which compose the small molecule binding sites. The detection of non-additive SAR is greatly facilitated by the synthesis of matrix libraries, an observation which has been described in detail elsewhere.[12]

All of the aforementioned compounds were synthesized as diastereomeric mixtures because the α-methyl phenoxyacetyl chlorides used in the library synthesis were commercially available only as racemates or were derived from racemic carboxylic acid precursors. We were interested in determining the influence of the α-methyl stereocenter on the potency and efficacy of these GHSR agonists, and so we performed a chromatographic separation of compound **8** to afford optically pure diastereomers **8a** and **8b**, as shown in Table 4.

The stereochemistry of the α-methyl group of the phenyoxyacetamide sidechain had a dramatic effect on GHSR potency, as illustrated by the 60-fold difference in EC_{50} and ⩾40-fold difference in K_i observed between the *R* (**8a**) and *S* (**8b**) isomers. The *R* stereochemistry for **8a** was assigned via X-ray crystallography, as shown in Figure 2.[13]

Table 5 describes similar stereochemical effects in the benzylamine series. In the case of the diastereomers of compound **9**, we observed that virtually all GHSR activity resided in **9b**, as **9a** was ⩾300-fold less active in both functional and binding assays. With **10**, the single diastereomer **10b** was 90-fold more potent than **10a** in the functional assay, and 70-fold more potent in the binding assay. Combined with the data from Table 4, it is clear that the stereochemistry of the α-methyl group of the acetamide sidechain makes a significant contribution to the potency of this chemotype.

Table 4
GHSR activity of diastereomers of compound **8**



8

Compound	Stereochem (*)	EC_{50} (nM)	% Control	K_i (nM)
8	*R* and *S*	30	90	260
8a	*R*	1200	100	⩾10,000
8b	*S*	20	100	250

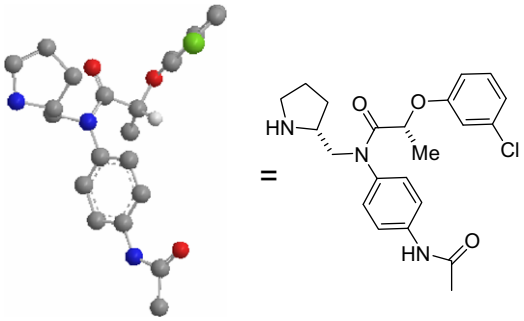
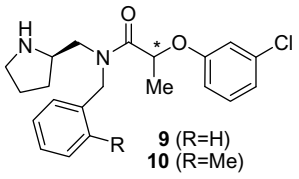


Figure 2. X-ray structure of **8a**.

Table 5
GHSR activity of diastereomers of compounds **9** and **10**



Compound	R	Stereochem (*)[a]	EC_{50} (nM)	% Control	K_i (nM)
9	H	*R* and *S*	7	90	300
9a	H	*R*	1500	100	⩾10,000
9b	H	*S*	4	90	30
10	Me	*R* and *S*	3	100	90
10a	Me	*R*	180	90	2200
10b	Me	*S*	2	100	30

[a] Stereochemistry was assigned by analogy to **8a** and **8b**.

In summary, this report describes the discovery and optimization of a potent series of GHSR agonists which were identified from a large GPCR-targeted screening library. Owing to the library pedigree of these screening hits, we were able to rapidly optimize micromolar partial agonists to full GHSR agonists with single-digit nanomolar potencies. In addition, the α-methyl stereochemistry of the phenoxyacetamide sidechain was found to have a dramatic effect on potency.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.120.

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13. X-ray coordinates for **8a** have been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 691452.