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Research paper

Optimization of the first small-molecule relaxin/insulin-like family peptide receptor (RXFP1) agonists: Activation results in an antifibrotic gene expression profile

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ABSTRACT

A dose responsive quantitative high throughput screen (qHTS) of >350,000 compounds against a human relaxin/insulin-like family peptide receptor (RXFP1) transfected HEK293 cell line identified 2-acetamido-*N*-phenylbenzamides **1** and **3** with modest agonist activity. An extensive structure-activity study has been undertaken to optimize the potency, efficacy, and physical properties of the series, resulting in the identification of compound **65** (ML-290), which has excellent in vivo PK properties with high levels of systemic exposure. This series, exemplified by **65**, has produced first-in-class small-molecule agonists of RXFP1 and is a potent activator of anti-fibrotic genes.

1. Introduction

The small-peptide hormone relaxin was discovered in 1926 and is primarily associated with pregnancy, due to its effects to relax pubic ligaments and soften the cervix to facilitate parturition. [1], [2] Since then it has been shown that blood concentrations of relaxin rise during the first trimester of pregnancy, stimulating cardiovascular and renal adjustments to accommodate the increased nutritional demands of the growing fetus and the elevated requirements for renal clearance of metabolic waste [3]. Relaxin production increases cardiac output, arterial compliance, renal blood flow and a decrease in systemic vascular resistance during pregnancy [4–6]. Both clinical and non-clinical studies using this hormone reinforce these cardiovascular effects in both males and females, which suggest the pharmacological utility of relaxin as a modulator of cardiovascular and renal functions in humans. [7], [8].

The target of relaxin is a group of G-protein coupled receptors, relaxin/insulin-like family peptide receptor (RXFP). [9], [10] Some of the physiological effects of relaxin are mediated by its interaction with RXFP1, which modulates several signal transduction pathways. [11], [12] Relaxin activation of RXFP1 up-regulates the endothelin system which leads to vasodilation. Activation also promotes extracellular matrix remodeling through regulation of collagen deposition, proliferation, cell invasiveness, and perhaps most importantly, overall tissue homeostasis. It also moderates inflammation by reducing levels of inflammatory cytokines such as TNF-α and TGF-β, as well as the induction of angiogenesis via the activation of transcription of vascular endothelial growth factor (VEGF) [13].

Understanding the biological effects of RXFP1 activation by relaxin has led to the evaluation of relaxin as a pharmacologic agent for the treatment of patients with acute heart failure (AHF),

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preeclampsia, and hypertensive disease. [6], [14] A recent phase 3 clinical trial utilizing recombinant human relaxin-2 (serelaxin) met its primary endpoint of improving dysponea through the fifth day in patients admitted for acute heart failure. [15], [16] We have previously reported the identification and evaluation of a series of small-molecule human relaxin agonists. [17], [18] Optimized compounds from this series are potent and highly selective activators of RXFP1 with similar efficacy to the natural hormone in functional assays. [19], [20] Using quantitative high-throughput screening, over 350,000 compounds from our Molecular Libraries Small Molecule Repository (MLSMR) were screened in an effort to identify small-molecule agonists of RXFP1 [21]. This small molecule library was created by the NIH to combine chemical resources from both academic and private institutions to increase chemical space diversity of compounds for a large variety of screening campaigns. In this disclosure we present the syntheses, SAR studies, and compound optimization which led to the identification of preclinical candidate **65**.

2. Results and discussion

The ability of these compounds to increase cyclic adenosine monophosphate (cAMP) levels in a human RXFP1 transfected HEK293 cell line was measured, and two compounds (**1** and **3**), both having a unifying molecular motif of 2-acetamido-*N*-phenylbenzamide were discovered (Fig. 1) [17].

The activity is reported through two parameters: EC_{50} (concentration necessary to reach 50% of maximum cAMP signal) and maximum response (efficacy indicated as the maximum observed increase in cAMP response), which were used to perform SAR analysis. Efficacies were normalized to forskolin, which had an EC_{50} of 47 nM and a defined maximum response of 100% [22].

Initial measurements of aqueous kinetic solubility of **1** and **3** (1.6 μg/mL, 3.7 μM and 0.9 μg/mL, 2.2 μM respectively) show poor solubility for these compounds. However, in assessing the stability of compounds **1** and **3** in mouse liver microsomes, compound **3** demonstrated very good stability after 60 min (70% parent remaining) versus 1 (2% parent remaining), so these data and the inherent problems associated with furans in mammalian metabolism led us to focus on the optimization of the cyclohexyl series based on compound **3** [23]. The general synthetic scheme to these is represented in Scheme 1.

Reagents and Conditions: For **3**–**22, 62**–**67**: (a) acid chloride, DCM, TEA, 0 °C to RT, 1–3 h; (b) substituted aniline or benzylamine, 2 M $AlMe_3$ in toluene, 100 °C, 16 h; (c) For **17**: **16**, DCM, MCPBA, RT, 12 h; (d) For **18**: **13**, phenylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, DMF, microwave irrad., 100 °C, 1 h; For **19**: **13**, 2-(trifluoromethylphenyl)boronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, DMF, microwave irrad., 100 °C, 1 h; For **20**: **13**, 3-(trifluoromethylphenyl) boronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, DMF, μW., 100 °C, 1 h; The first series of compounds 3–22 (Table 1) were prepared in a straightforward manner from 2 (see Experimental Standard Procedures), with the first amide formation performed by reaction with cyclohexylcarbonyl chloride followed by subsequent AlMe3 mediated

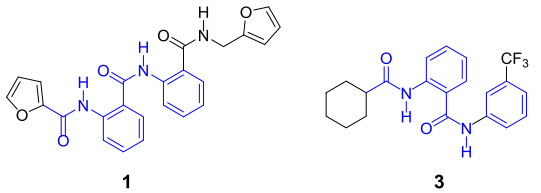
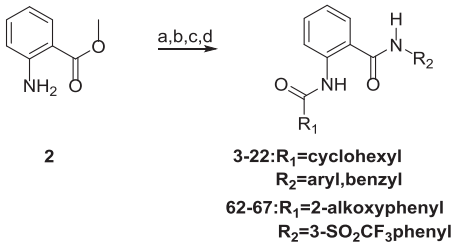
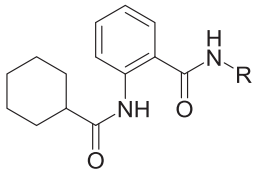


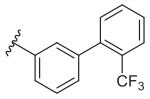
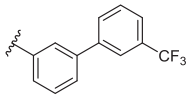
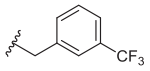
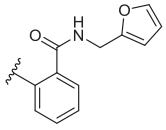
Fig. 1. Initial RXFP1 hits from the Molecular Probe Center Network Library.



Scheme 1. Synthesis of the Western Cyclohexyl and Optimized Analogues.

Table 1
SAR of the cyclohexyl-2-acetamido-*N*-phenylbenzamide Series.



Cmpd	R	EC_{50} (μM)[a]	Max. Response[b]
3	3-trifluoromelhylphenyl	1.88	92%
4	4-trifluoromethylphenyl	94.0	46%
5	phenyl	94.0	57%
6	2-methylphenyl	inactive	N/A
7	3-methylpheny!	37.4	65%
8	4-methyphenyl	187	32%
9	3-*tert*-butlyphenyl	2.66	70%
10	3-nitrophenyl	5.93	93%
11	3-fluorophenyl	13.3	81%
12	3-chlorophenyl	3.34	89%
13	3-bromophenyl	2.65	91%
14	3-methoxy phenyl	37.4	74%
15	3-thiomelhylphenyl	5.29	84%
16	3-trifluoromelhylhiophenyl	1.88	90%
17	3-trifluoromethylsulfonylphenyl	1.06	87%
18	3-biphenyl	inactive	N/A
19		2.65	74%
20		inactive	N/A
21		inactive	N/A
22		9.40	45%

[a] The EC50 is expressed in micromolar and is the concentration necessary to reach 50% of maximum cAMP signal.
[b] The maximum response is the efficacy indicated as the maximum observed increase in cAMP response.

second amide formation with the appropriate aniline or amine. This direct amidation using an aniline and an ester proved to be robust and broad in scope. Compounds 18–20 were prepared from bromo-compound 13 using standard Suzuki conditions.

Table 1 discloses the initial results of our SAR studies. It is clear upon viewing the data that the 3-position of the pendant R-phenyl