Journal of Medicinal Chemistry Letter

Discovery and Optimization of Substituted 1-(1-Phenyl-1*H*-pyrazol-3-yl)methanamines as Potent and Efficacious Type II Calcimimetics

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Received August 17, 2009

Abstract: Our efforts to discover potent, orally bioavailable type II calcimimetic agents for the treatment of secondary hyperparathyroidism focused on the development of ring constrained analogues of the known calcimimetic R-568. The structure–activity relationships of various substituted heterocycles and their effects on the human calcium-sensing receptor are discussed. Pyrazole **15** was shown to be efficacious in a rat in vivo pharmacodynamic model.

Secondary hyperparathyroidism (sHPT^{*a*}) is a chronic, debilitating condition that typically afflicts people with chronic kidney disease (CKD).^{1,2} The disease is characterized by persistently high levels of circulating parathyroid hormone (PTH). The dysregulation of PTH consequently causes an imbalance in extracellular calcium ion (Ca²⁺) levels. If left untreated, sHPT ultimately results in abnormal mineral metabolism that can lead to severe complications such as osteodystrophy and tissue calcification.³

In efforts to find treatments to alleviate sHPT, researchers have sought to develop small molecule based drugs that target the metabolic pathways associated with PTH secretion. A crucial discovery in this field was made by Brown and co-workers in 1993 when they cloned and characterized the calcium-sensing receptor (CaSR) from bovine parathyroid gland.⁴ The CaSR, a member of the G-protein coupled receptor (GPCR) family, is expressed mainly on the surface of chief cells in the parathyroid gland and is involved in the regulation of extracellular levels of calcium ions (Ca²⁺).⁵ CaSR controls Ca²⁺ levels by acting as a negative regulator of PTH secretion. When extracellular Ca^{2+} levels drop, the activity of the CaSR is reduced, resulting in increased PTH secretion. The increased PTH levels, in turn, elevate extracellular Ca²⁺ levels by stimulating calcium resorption from the bone and calcium reabsorption from the kidney filtrate. Consequently, the increase in extracellular Ca²⁺ levels activate the CaSR which then signals to the chief cells to stop releasing PTH.



Figure 1. Proposed constrained analogues of 1.

The CaSR senses calcium ions through a functional flytraplike extracellular domain.^{6,7} Type I calcimimetics, such as Ca²⁺, polyarginine, and other polycationic species, bind to this moiety and elicit a classic agonistic response.⁸ Agents that interact with the CaSR enzyme at sites distinct from the Ca²⁺ binding site and sensitize the receptor to calcium ions are called type II calcimimetics.⁹ Such positive allosteric modulators for the CaSR enzyme have recently been the focus of research toward novel therapies for sHPT.^{10–13} Cinacalcet HCl (Sensipar, Mimpara, Figure 1), a type II calcimimetic, has been approved for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease on dialysis as well as the treatment of hypercalcemia in patients with parathyroid carcinoma.¹⁴

Herein we report our efforts to develop a more efficacious and metabolically stable alternative to the cinacalcet analog R-568 (1).¹⁵ Although shown to be efficacious at decreasing PTH levels in human patients,¹⁶ advancement of 1 was discontinued because of its poor metabolic profile.¹⁷ We hypothesized that reducing the number of rotatable bonds would lead to compounds with improved potency and pharmacokinetic characteristics (Figure 1).¹⁸ The structure–activity relationship (SAR) studies focused on the substitution of the propyl linker with various five-membered heterocycles.

The chemical syntheses of the ring constrained analogues of 1 required different synthetic sequences for each heterocyclic scaffold (Scheme 1). The imidazole derivatives, 2 and 3, were assembled by a reductive amination with a functionalized aldehyde and (R)-methylbenzylamine. The thiazole and oxazole analogues 4-6 were prepared by reductive amination with the appropriate aldehyde and amine or via radical benzylic bromination and subsequent alkylation with (R)-methylbenzylamine. Pyrazole derivative 7 was synthesized via N-arylation of methyl pyrazole-3-carboxylate, followed by conversion to the corresponding aldehyde using a sequential reduction/oxidation protocol. Reductive amination of the resulting aldehyde then afforded the desired pyrazole analogue 7.

These constrained heterocyclic analogues were tested in a cell-based functional assay consisting of HEK293 cells that expressed human CaSR. Induced Ca²⁺ flux, resulting from the activation of the CaSR, was measured using a FLIPR based protocol. Results of the in vitro profiling of the initial set of ring constrained analogues of 1 are summarized in Table 1. Of the six five-membered heterocycles tested, 2-phenylpyrazole 7 (EC₅₀ = $1.5 \,\mu$ M) was the only derivative with activity at compound concentrations lower than 5 μ M.

Given the promising potency of 7, optimization of this scaffold was subsequently pursued. In addition to improving potency, efforts were made to limit potential sites of metabolism. These liabilities could include the benzylic methylene moiety and the three aromatic rings. To this end, pyrazole

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^{*a*} Abbreviations: sHPT, secondary hyperparathyroidism; CKD, chronic kidney disease; PTH, parathyroid hormone; CaSR, calcium-sensing receptor.

Scheme 1. Syntheses of Heterocycles $2-7^a$



^{*a*} Reagents and conditions: (a) MeI or SEMCl, $C_{s_2}CO_3$, DMF, room temp; (b) (i) *n*-BuLi, -40 °C, THF; (ii) DMF, -78 °C to room temp; (c) (*R*)-1-phenylethanamine, NaBH(OAc)₃, AcOH, DCE, room temp; (d) MeOH/concentrated HCl (2:1), 40 °C; (e) AcCl, pyridine, CH₂Cl₂, -78 °C to room temp; (f) Lawesson's reagent, THF, 50 °C (X = S); (g) NCS, AcOH, CCl₄, reflux; (h) NBS, AIBN, CCl₄, reflux. (i) (*R*)-1-phenylethanamine, THF, rt. (j) Pd/C, H₂, MeOH, rt. (k) trans-*N*,*N*'-dimethyl-1,2-cyclohexanediamine, CuI, K₃PO₄, iodobenzene, toluene, 110 °C; (l) (i) LiAlH₄, THF, 0 °C; (ii) PCC, CH₂Cl₂, room temp.

^b 2-Methyl-5-phenyloxazole is commercially available.

Table 1. In Vitro Activities of Preliminary Heterocyclic Analogues

compd	А	В	С	D	$\begin{array}{c} \text{CaSR} \\ \text{EC}_{50} \left(\text{nM} \right)^{a} \end{array}$
1					194 ± 30
2	Ν	С	CH	NH	> 5000
3	Ν	С	CH	NMe	> 5000
4	S	С	Ν	CH	> 5000
5	Ν	С	CH	Ο	> 5000
6	Ν	С	CH	S	> 5000
7	Ν	Ν	CH	CH	1580 ± 743

^{*a*} Values expressed as the mean \pm SD with $n \ge 3$.

derivatives containing substitutions at these sites of interest were synthesized and profiled.

The syntheses of analogues containing alkyl substituents at the benzylic position are described in Scheme 2. The route utilized a Weinreb amide intermediate, Grignard addition, and subsequent asymmetric reductive amination. Reductive amination of the functionalized acetophenones with chiral methylbenzylamines produced the anti-diastereomer as the major product.^{19,20} This stereochemical preference was confirmed by an X-ray crystal structure of compound **15** (see supporting info).

Next, we explored the effects of substitution on the pyrazole ring and the adjacent phenyl ring. The synthesis of the methoxypyrazole derivatives 13-18 began with the assembly of a pyrazolinone ester intermediate from the corresponding aryl hydrazide (Scheme 2).²¹ Subsequent Mitsunobu alkylation with methanol and derivatization delivered the desired compounds.

The SAR around the pyrazole scaffold is shown in Table 2. Modification of **4** to include a benzylic methyl group ($R_1 = Me$) and a trifluoromethyl group ($R = CF_3$) to the phenyl ring provided **8**, which demonstrated an approximately 6-fold increase in activity compared to **7** ($EC_{50} = 233$ nM). Larger alkyl groups at the benzylic center (R_1) proved to be deleterious to activity (**9**, **10**). Incorporation of a halogen on the benzylamine fragment (e.g., **11** and **12**) resulted in a further 2- to 3-fold increase in activity. Yet, another modest increase in activity was observed when a methoxy group was incorporated onto the pyrazole ring (**13**). Further exploration revealed that other lipophilic groups were well tolerated with the 4-Br analogue demonstrating the highest activity (**15**, $EC_{50} =$ 23 nM). Attempts to incorporate polar functionalities (**17**, **18**) resulted in a significant decrease in potency. Scheme 2. Synthesis of Substituted Pyrazoles $(8-18)^a$



^{*a*} Reagents and conditions: (a) *N*,*O*-dimethylhydroxylamine hydrochloride, HATU, DIEA, DMF, room temp; (b) R₁MgBr, THF, 0 °C; (c) arylamine, titanium(IV) isopropoxide, NaBH₄, THF, room temp; (d) dimethyl acetylenedicarboxylate, NaOMe, MeOH, room temp; (e) triphenylphosphine, DIAD, MeOH, room temp; (f) LiOH, THF, water, 60 °C; (g) MeMgBr, THF, 0 °C.

 Table 2. In Vitro Activities of Substituted Pyrazole Derived Calcimimetics



compd	Ar	R	R_1	R_2	$\begin{array}{c} \text{CaSR} \\ \text{EC}_{50} \left(\text{nM} \right)^{a} \end{array}$
7	Ph	Н	Н	Н	1580 ± 743
8	Ph	$4-CF_3$	Me	Н	233 ± 134
9	Ph	$4-CF_3$	Et	Η	1180 ± 582
10	Ph	$4-CF_3$	iPr	Н	2340 ± 1810
11	3-F-Ph	$4-CF_3$	Me	Η	148 ± 64
12	3-Cl-Ph	$4-CF_3$	Me	Н	92 ± 38
13	Ph	$4-CF_3$	Me	MeO	70 ± 66
14	3-Cl-Ph	$4-CF_3$	Me	MeO	41 ± 30
15	3-Cl-Ph	4-Br	Me	MeO	23 ± 3
16	3-Cl-Ph	3-Cl	Me	MeO	57 ± 58
17	3-Cl-Ph	4-CO ₂ H	Me	MeO	1920 ± 1550
18	3-Cl-Ph	4-tetrazole	Me	MeO	> 5000

^{*a*} Values expressed as the mean \pm SD with $n \ge 4$.

Given the potent in vitro data, **15** ($EC_{50} = 23$ nM) was selected for further evaluation. Analogue **15** demonstrated a favorable pharmacokinetic profile in rats (Table 3). In comparison to **1**, **15** possessed significantly improved clearance (CL), mean residence time (MRT), and bioavailability (% *F*).

On the basis of the promising characteristics of **15**, this molecule was progressed to a rat pharmacodynamic (PD) model. In this study, the compound was administered orally to male Sprague–Dawley rats to determine its effects on PTH levels (Figure 2). Pyrazole **15**, at 3 mg/kg po (solid red line), lowered PTH to a greater extent than **1** at 30 mg/kg po (dotted blue line). Specifically, **15** reduced PTH to the lower limit of

Table 3. Pharmacokinetic Profiles for 1 and Pyrazole 15 in Male Sprague–Dawley Rats



compd	CL ((L/h)/kg) ^a	$V_{\rm ss}$ $({\rm L/kg})^a$	MRT (h) ^a	$\begin{array}{c} \text{AUC} \\ (\text{ng} \cdot \text{h}/\text{L})^b \end{array}$	$C_{\max} (ng/L)^b$	F $(\%)^b$
1	3.9	6.8	1.74	22	4.2	5
15	1.3	7.0	11.8	444	36	24

 a 0.5 mg/kg intravenous dose (100% solution in DMSO). b 2 mg/kg oral dose (0.5% methyl cellulose/1% Tween 80 in water).



Figure 2. Effects of 1 and 15 on the PTH levels in Sprague–Dawley rats.

detection (< 3 pg/mL) in 4 of 5 animals at 1 h post dose and in all (5 of 5) animals at 4 and 8 h.²²

In conclusion, a novel series of pyrazole-based type II calcimimetics have been discovered. Ring constrained derivatives of 1 were synthesized as a method to improve potency and the pharmacokinetic profile. This strategy led to the development of a highly potent, orally bioavailable compound that significantly lowered PTH levels in a rat in vivo PD model.

Acknowledgment. The authors thank Rashid Syed for providing X-ray crystallographic support, Chris Wilde for spectroscopic assistance, and Guifen Xu, Jie Chen, Dean Hickman, Ronya Shatila, Anna Akrami, and Valerie Almon for pharmacokinetic analysis.

Supporting Information Available: Experimental details, characterization methods and results, and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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