Bioorganic & Medicinal Chemistry Letters 19 (2009) 3328–3332

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The discovery of novel calcium sensing receptor negative allosteric modulators

Gayatri Balan, Jonathan Bauman, Samit Bhattacharya, Mayda Castrodad, David R. Healy, Michael Herr, Paul Humphries, Sandra Jennings, Amit S. Kalgutkar, Brendon Kapinos, Vishal Khot, Kimberly Lazarra, Mei Li, Yan Li, Constantin Neagu, Robert Oliver, David W. Piotrowski *, David Price *, Hong Qi, Holly A. Simmons, James Southers, Liuqing Wei, Yan Zhang, Vishwas M. Paralkar

Pfizer Global Research and Development, Groton/New London Laboratories, Pfizer Inc., Eastern Point Road, Groton, CT 06340, USA

ARTICLE INFO

Article history:
Received 5 March 2009
Revised 6 April 2009
Accepted 8 April 2009
Available online 18 April 2009

Keywords:
Calcium sensing receptor
Negative allosteric modulators
Osteoporosis
Parathyroid hormone

ABSTRACT

The design and profile of a series of zwitterionic calcium sensing receptor negative allosteric modulators is described. Evaluation of key analogues using a rat model demonstrate a robust response, significantly improved potency over ronacaleret and have the potential as an oral, anabolic treatment for osteoporosis.

From 2000 to 2005 in the United States the number of patients greater than 55 years old seeking treatment for osteoporosis has increased by 10%. This increase is clearly linked to the improved longevity of the population.[1] Currently the majority of patients are treated with antiresorptive therapy of which the bisphosphonate class are the most commonly prescribed. Alendronic acid (marketed as Fosamax™) is the market leader and is now available through generic manufacturers.[2]

Patients who either fail or are intolerant to antiresorptive therapy used to have limited alternatives. However, when teriparatide (marketed as Forteo™), a recombinant form of parathyroid hormone (PTH) was introduced in 2002, anabolic therapy became an option for those patients with severe osteoporosis. Teriparatide is the 1–34 amino acid portion of the complete human parathyroid hormone molecule that contains amino acid sequence 1–84.[3] Endogenous PTH is the primary regulator of calcium and phosphate metabolism in bone and kidney. Daily subcutaneous injections of teriparatide stimulate new bone formation leading to increased bone mineral density. However, it is known that sustained increase of PTH levels can lead to increased bone resorption and so have an opposite effect to that needed within the osteoporosis arena.[4] Although, sales of teriparatide have increased since its launch its use is limited due to a restricted label and the necessity for an administration route via injection. Therefore there is still a large commercial opportunity for an orally available bone anabolic agent that can be used more widely in the treatment and prevention of osteoporosis.

Rather than focusing resource into delivering an orally available form of the peptide PTH an alternative approach is to deliver a traditional small molecule that could stimulate the release of PTH endogenously. The calcium-sensing receptor (CaSR) is a class C G-protein coupled receptor primarily located on the surface of parathyroid cells that senses extracellular levels of calcium ion levels and increases release of PTH when calcium levels are low.[5] Negative allosteric modulators of the calcium receptor (also known as calcilytics) are therefore able to stimulate the release of PTH.[6] It is important to note that these CaSR negative allosteric modulators must be short acting to stimulate a pulsatile release of PTH. In order to mimic the short half life of endogenous PTH or teriparatide, these agents should have a pharmacokinetic half life of approximately one hour. This knowledge has been key to the medicinal chemistry design of the calcilytics throughout all research groups, in particular the pharmacokinetic properties of the small molecule. Assuming that the free concentration of the negative allosteric modulator drives the release of the PTH, this necessitates that the compounds have a rapid onset of action (short T_{max} after oral administration) and a sufficiently high clearance and/or low volume of distribution so that the drug is cleared rapidly. It is also important to deliver a compound that is not susceptible to interpatient variability through drug-drug interactions or has a risk of accumulation because these effects could lead to safety concerns due to hypercalcemia. This is a difficult pharmacokinetic profile to predict preclinically and so a multiple clinical candidate strategy

* Corresponding authors.
E-mail address: david.a.price@pfizer.com (D. Price).

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doi:10.1016/j.bmcl.2009.04.044

was adopted. A flurry of recent publications, patents and disclosures on clinical compounds has prompted us to report our research into this highly competitive arena.[7]

The first orally bioavailable small molecule calcilytic reported was NPS-2143, however, this compound was hampered by hERG and P450 inhibition and inappropriate pharmacokinetics due to a long half life.[8] The most advanced compound in the clinic was ronacaleret which had reached Phase 2 by the start of 2008, Figure 1.[9] The structural similarities between NPS-2143 and ronacaleret are clear and the addition of the carboxylic acid moiety could improve both the off target pharmacology issues associated with NPS-2143 and also modulate the pharmacokinetic half life. Ronacaleret has shown insufficient efficacy in clinical trials and development has been terminated. Clearly this lack of efficacy could be due to a variety of factors such as poor exposure, limited ability to dose escalate due to safety concerns or simply due to a lack of confidence in the mechanism. The confidence in the mechanism within the clinical setting was reestablished shortly after with the disclosure that JTT-305 (also known as MK-5442) had shown a positive proof of concept in a 12 week Phase 2a study. The structure of JTT-305 is unknown but is believed to be in a similar chemotype to that of ronacaleret.

Taking ronacaleret as the project lead the ability of ronacaleret to increase endogenous PTH secretion was evaluated in Sprague–Dawley male rats following oral administration. The plasma concentration of PTH1-84 was measured by using a commercially available PTH ELISA kit which detects the full length of rat PTH1-84 specifically. No PTH spike was discernible upon oral administration of ronacaleret at 10 mg/kg (suspension in 0.5% methylcellulose) despite achieving a reasonably high total systemic exposures ($C_{max} \sim 300$ nM); the free concentration of ronacaleret was therefore <3 nM. In a parallel study the oral bioavailability of ronacaleret in rats was determined to be 12%. The IC_{50} of ronacaleret against the rat CaSR receptor expressed in HEK cells was determined to be 320 nM. The conclusion was that for compounds with equivalent pharmacokinetic properties a significant increase in potency would be required to deliver an anticipated in vivo efficacy signal of PTH elevation. Therefore, it was decided that an initial goal of identifying significantly more potent compounds than ronacaleret was an appropriate project starting point. In our hands the IC_{50} of ronacaleret against the human CaSR receptor expressed in HEK cells was determined to be 150 nM, which was within threefold of the rat potency. Previous experience of working within the amino-alcohol series had shown a consistent relationship between rat and human potencies (<threefold variation). We did not foresee species differences in potency to be a major concern in project prosecution and this was indeed the case. For the sake of expediency only rat CaSR data was generated for compounds that we were going to progress to in vivo studies to measure the PTH response.

Profiling of ronacaleret through a battery of binding assays showed no significant activity against a range of pharmacologically relevant enzymes, ion channels and receptors up to 10 μM, as measured in binding competition and functional assays. Further profiling for cardiac effects and in particular those mediated via hERG showed only very weak affinity using whole cell voltage clamp techniques with <10% inhibition at 10 μM. We were confident that by staying within the amino-acid expression we would have an opportunity to deliver a safe and efficacious agent providing we could improve the potency against the human and rat CaSR by approximately an order of magnitude.

A review of the available literature suggested that the carboxylic acid was tolerated in a variety of vectors attached to the phenoxy motif. Indeed, using in silico structural overlays, it was impossible to have the carboxylic acid overlap from the differing expressions within this chemotype while maintaining the positions of the basic center and hydroxyl groups. When NPS-2143 and ronacaleret were profiled in our laboratories it appeared that NPS-2143 was significantly more potent as a negative allosteric modulator of the CaSR. This confirmed that the acid expression is not delivering a key interaction with the receptor, rather it is modifying the overall physio-chemistry of the molecule. Our initial strategy was to retain the acid expression and then deliver novel, inventive methods of attaching the carboxylic acid to the amino-alcohol template. Comparing the human microsomal stability of NPS-2143 and ronacaleret it was clear that the acid expression has also significantly reduced the lipophilicity of the series and delivered a significant improvement in human microsomal stability, Table 1. Both NPS-2143 and ronacaleret have an equivalent free fraction in the microsomal preparation so this is not driving the difference in stability. The disconnect between rat and human liver microsomal stability for ronacaleret was concerning and difficult to find a strong rationale for. This data gave us the opportunity to moderately increase the lipophilicity of the series while retaining the microsomal stability and potentially improving potency through hydrophobic interactions with the receptor. We were also aware that there were many other non P450 mediated clearance pathways available to lipophilic amino-acids, and it was decided to progress compounds aggressively to in vivo studies as decision making experiments.

In order to meet these design criteria, the team was intrigued at the utility of palladium mediated, sequential reactions on norbornadiene as illustrated in Scheme 1. Not only is the starting material readily available, but the hydroarylation reaction is tolerant of a variety of differing aryl functionalities giving us synthetic flexibility in the preparation of analogues.[10] Indeed, both the cyclopropanation and hydroarylation reactions can be safely performed on multigram quantities. It was decided that for the sake of speed the project would screen the final mixture of diastereoisomers and then for compounds of interest separate the diastereoisomers for in vivo profiling.

We were delighted to find that the compounds prepared compared favorably in terms of potency with compound **1** being only twofold less potent than NPS-2143. Progression of **3** to microsomal preparations also suggested that in this molecular expression we had similar clearance rates between rat and human microsomes which gave us hope that we would be able to predict our clinical dose with an improved degree of confidence. We were aware that within this series we working at the higher end of accepted physiochemical space for orally bioavailable drugs. There have been numerous analysis' investigating molecular properties that influence oral bioavailability and it is generally agreed that molecular

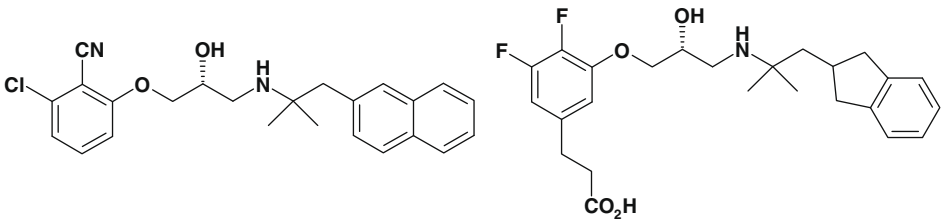
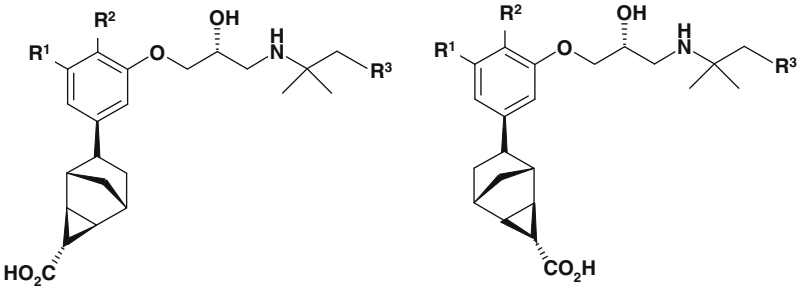
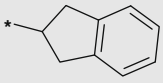
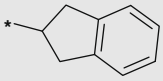
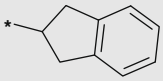
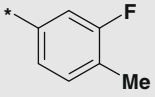
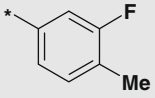
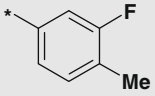
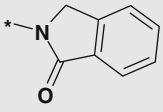


Figure 1. Structure of NPS-2143 and ronacaleret.

Table 1
Comparison of potencies and microsomal stability of phenoxy analogues

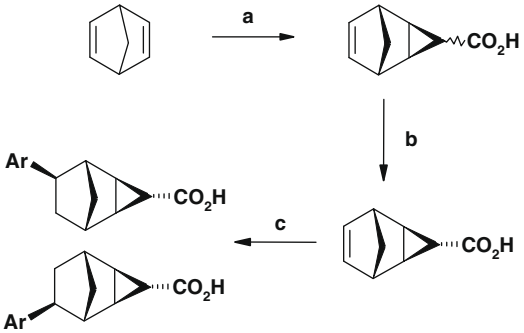


Compounds[a]	R^1	R^2	R^3	h-CaSR (IC_{50}, nM)[b]	HLM (clint)[c]	RLM (clint)[d]	MWt	*c*log *P* (log *D*)
NPS-2143				23	157	>100	409	5.1
Ronacaleret				146	<8	90	448	2.1 (1.5)
1	F	F		56	19	35	526	3.7 (2.6)
2	H	H		900	15	50	478	3.5
3	H	CN		6	17	25	515	3.3 (2.0)
4	H	H		3500	23	38	470	3.3
5	H	F		300	20	Not done	499	3.4
6	H	CN		23	15	27	507	3.0 (2.3)
7	H	CN		350	9	<10	530	1.5

[a] All compounds are a 50:50 mixture of two diastereoisomers as drawn.
[b] Potency at human recombinant CaSR recpetore expressed in HEK cells. All assay determinations $n = 3$.
[c] Human liver microsome stability expressed as intrinsic clearance (μL/min/mg of microsomal protein).
[d] Rat liver microsome stability expressed as intrinsic clearance (μL/min/mg of microsomal protein).

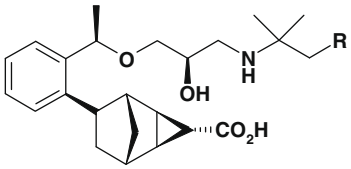
weight and hydrogen bonding propensity are key components.[11] We were keen that any further manipulations within this series did not further compromise these characteristics. Incorporating the 2-cyano group generated compound **3** where we had achieved high potency, acceptable microsomal stabilities and physiochemical profile. Attempts to improve upon compound **3** through alternative expressions to the indane were unsuccessful with compound **6** being the most interesting profiled.

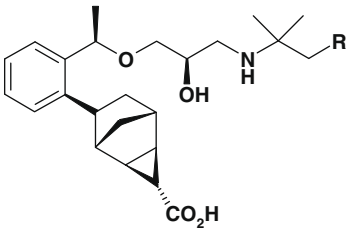
Comparing compounds **3** and **6** from their in vitro profile, they were difficult to differentiate except that compound **3** had a reproducibly improved potency against the CaSR. It was decided to separate the diastereoisomers by careful chromatography and perform rat in vivo pharmacokinetic studies on the individual diastereoisomers. Interestingly, the separated diastereoisomers were equipotent at the CaSR receptor again confirming our hypothesis that the carboxylic acid is not undergoing a key interaction with the

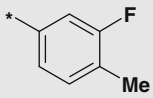
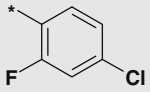
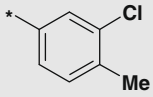
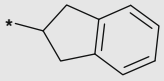


Scheme 1. Synthesis of cyclopropyl acids; Reagents and conditions: (a) ethyldiazoacetate, $PdCl_2$, hexanes, 60 °C; (b) NaOMe, IMS, rt then conc. H_2SO_4, EtOH, rt; (c) ArI, $Pd(OAc)_2$, DMSO, Et_3N, P(*o*-tol)$_3$, HCO_2H, 75 °C.

Table 2
Comparison of potencies and microsomal stability of benzyloxy analogues





Compounds[a]	R	h-CaSR (IC_{50}, nM)[b]	HLM (clint)[c]	RLM (clint)[d]	MWt	clog *P* (log *D*)
8		25	35	45	510	3.5 (2.4)
9		50	16	14	530	3.7 (2.8)
10		18	33	Not done	526	4.1 (2.6)
11		3500	23	38	470	3.7

[a] All compounds are a 50:50 mixture of two diastereoisomers as drawn.
[b] Potency at human recombinant CaSR recpetore expressed in HEK cells. All assay determinations $n = 3$.
[c] Human liver microsome stability expressed as intrinsic clearance (μL/min/mg of microsomal protein).
[d] Rat liver microsome stability expressed as intrinsic clearance (μL/min/mg of microsomal protein).

receptor in terms of binding energy. Following iv administration (1 mg/kg) for each diastereoisomer the volume of distribution was moderate for both (V_d = 5 L/kg), however, with a clearance of approximately liver blood flow (70 and 83 mL/min/kg) the measured half life of the diastereoisomers was 1.5 h. An oral leg was performed (10 mg/kg), and the oral bioavailability was poor for both diastereoisomers (<10%) due to the high clearance. The experiment was valuable as it directed the team to move away from the phenoxy template and seek alternative expressions for the acid moiety where our in vitro assays were more predictive of in vivo outcomes. The in vitro/in vivo clearance disconnect is not entirely surprising as the microsomal preparation will only significantly predict for the P450 mediated oxidation component of the in vivo clearance. Within this series the opportunities for a range of other in vivo clearance mechanisms such as glucuronidation and organic anion/cation transporters is high and with our current knowledge of these systems an accurate prediction of in vivo clearance is impossible.[12] This confirmed our desire within the project to drive compounds as rapidly as possible to an in vivo assessment of pharmacokinetics rather than relying on in vitro assays alone.

A reassessment of the literature suggested that the phenoxy expression could be replaced by a benzyloxy motif.[13] This was highly attractive as we could again use the hydroarylation methodology and reuse phenethyl intermediates previously prepared. This enabled the rapid identification of a number of interesting compounds, Table 2. Again these compounds are of equivalent potency to ronacaleret and the correlation between human and rat microsomal stability was good except for one outlier, compound **11**. We have no clear rationale as to why there should be a significant difference between the microsomal stabilities in human and rat for compound **11**. From these compounds it was decided to progress compound **8** as the strongest representative of the series into in vivo rat pharmacokinetic studies. After separation of the diastereoisomers we were delighted to find that after oral administration (10 mg/kg) a diastereoisomer showed high oral bioavailability (>60%) and a total plasma C_{max} of 1 μM. With this drug concentration we were confident that this was a compound of quality to progress to in vivo efficacy studies. Advancing **8** to a PTH secretion experiment in Sprague–Dawley male rats using oral administration (10 mg/kg) the compound displayed a robust PTH response comparable to literature agents. Further progression through a panel of screens to assess off target pharmacology showed no significant activity <10 μM and no activity at the hERG channel or P450 enzymes. With this improvement over both NPS-2143 and ronacaleret in terms of both efficacy and off target pharmacology the compound was profiled in an in vivo toleration study. Pleasingly it showed no significant adverse effects at the 500 mg/kg dose used in the 4 day rat study and was accepted for progression into preclinical studies.

In conclusion we have described the project strategy and design criteria we used for the discovery of a calcylitic using a simple yet powerful synthetic methodology. The compound displays robust efficacy in a standard rodent model that is routinely used in the osteoporosis arena. It also has appropriate pharmacokinetics in rat with good oral bioavailability and is well tolerated in preliminary in vitro and in vivo safety studies. Further work in this highly attractive mechanism will be described in following publications.

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