Bioorganic & Medicinal Chemistry Letters 23 (2013) 6625–6628

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Metabolism-guided discovery of a potent and orally bioavailable urea-based calcimimetic for the treatment of secondary hyperparathyroidism

Paul M. Wehn [a], Paul E. Harrington [b,*], Timothy J. Carlson [c], James Davis [d], Pierre Deprez [e], Christopher H. Fotsch [b], Mark P. Grillo [c], Jenny Ying-Lin Lu [f], Sean Morony [d], Kanaka Pattabiraman [a], Steve F. Poon [b], Jeff D. Reagan [f], David J. St. Jean Jr. [b], Taoues Temal [e], Minghan Wang [d], Yuhua Yang [f], Charles Henley III [d], Sarah E. Lively [a]

[a] Department of Medicinal Chemistry, Amgen Inc., 1120 Veterans Blvd., South San Francisco, CA 94080, USA
[b] Department of Medicinal Chemistry, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[c] Department of Pharmacokinetics and Drug Metabolism, Amgen Inc., 1120 Veterans Blvd., South San Francisco, CA 94080, USA
[d] Department of Metabolic Disorders, Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA 91320, USA
[e] Galapagos SASU, 102 Avenue Gaston Roussel, 93230 Romainville, France
[f] Department of Metabolic Disorders, Amgen Inc., 1120 Veterans Blvd., South San Francisco, CA 94080, USA

ARTICLE INFO

Article history:
Received 3 September 2013
Revised 22 October 2013
Accepted 23 October 2013
Available online 31 October 2013

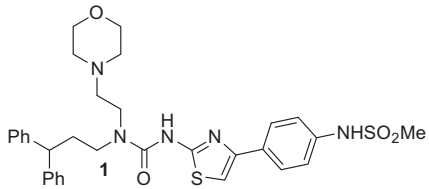
Keywords:
Calcimimetics
Positive allosteric modulators
1,2,4-Thiadiazoles
Metabolite ID
Time dependent inhibition

ABSTRACT

A series of urea based calcimimetics was optimized for potency and oral bioavailability. Crucial to this process was overcoming the poor pharmacokinetic properties of lead thiazole **1**. Metabolism-guided modifications, characterized by the use of metabolite identification (ID) and measurement of time dependent inhibition (TDI) of CYP3A4, were essential to finding a compound suitable for oral dosing. Calcimimetic **18** exhibited excellent in vivo potency in a 5/6 nephrectomized rat model and cross-species pharmacokinetics.

Secondary hyperparathyroidism (secondary HPT) is a condition characterized by the chronic elevation of parathyroid hormone (PTH) that often develops in patients with compromised kidney function.[1] The regulation of PTH levels is governed by the calcium-sensing receptor (CaSR), a class 3 G protein-coupled receptor (GPCR) primarily expressed on the parathyroid gland.[2] Increases in serum Ca^{2+} concentration raise the level of activation of the CaSR and inhibit PTH secretion, whereas decreases in serum Ca^{2+} concentration reduce CaSR activation and enhance PTH secretion.[3] Left untreated, secondary HPT can lead to limb deformities as well as bone and joint pain. The discovery of positive allosteric modulators of the CaSR, type II calcimimetics, represents a novel therapy for the treatment of secondary HPT.[4] Such agents increase the sensitivity of the CaSR to serum Ca^{2+} and thereby decrease secretion of PTH.

In a groundbreaking publication, Nemeth and co-workers described the in vitro and in vivo pharmacology of the first type II calcimimetic with the disclosure of *R-568* and related analogues.[5] Subsequent work to identify new calcimimetic agents has focused almost entirely on derivatives that retain a basic α-methylbenzyl amine moiety.[6] A novel series of benzothiazole urea based calcimimetics arising from *R-568* and fendiline was recently disclosed.[7] Further development of this series led to **1**.[8] In vivo studies with **1**, using a 5/6 nephrectomized rat model of kidney failure wherein



hCaSR EC_{50} = 16 nM
Rat CL = 3.8 L/h/kg

Figure 1. Thiazole **1**, a urea-based calcimimetic.

* Corresponding author. Tel.: +1 805 313 5564; fax: +1 805 480 1337.
E-mail address: pharring@amgen.com (P.E. Harrington).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.10.050

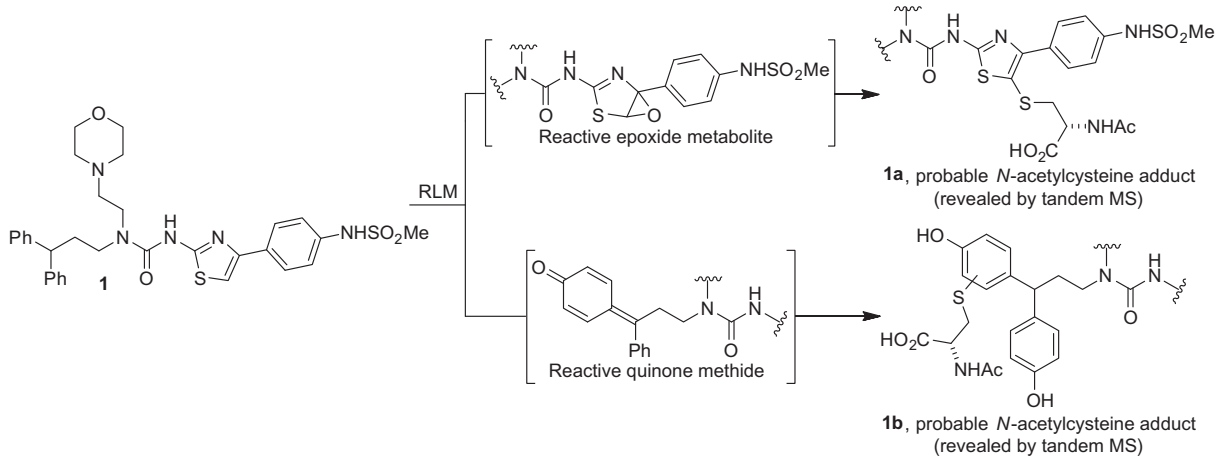
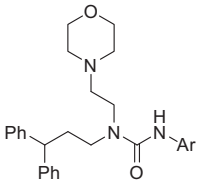
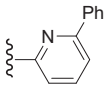
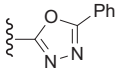
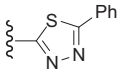
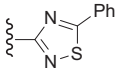
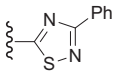


Figure 2. Metabolites of **1** formed from incubation with rat liver microsomes (RLM) and elucidated by tandem mass spectrometry.

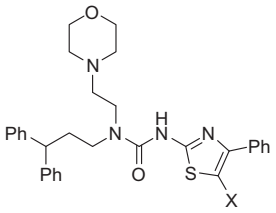
Table 1
Heterocyclic replacements of thiazole



Compound	Ar	hCaSR EC_{50} (nM)[a]
2		300
3		1030
4		286
5		175
6		23

[a] Values are an average of at least two determinations.

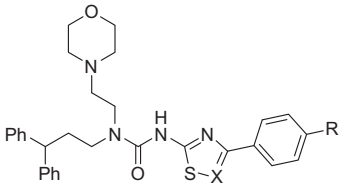
Table 2
Impact of electron withdrawing groups on TDI potential



Compound	X	%3A4 Activity Remaining[a]	hCaSR EC_{50} (nM)[b]
7	H	22	36
8	F	28	53
9	SO_2Me	59	17
10	CN	76	157
11	Cl	91	18

[a] CYP3A4 experiments were performed as previously described Ref. 11
[b] Values are an average of at least two determinations.

Table 3
Chlorothiazole and thiadiazole analogues of **1**



Compound	X	R	hCaSR EC_{50} (nM)[a]	PK Data[b] CL (L/h/kg)/%F
12	CCl	$NHSO_2Me$	3	1.4/37
13	CCl	SO_2NH_2	2	0.42/16
14	N	$NHSO_2Me$	6	4.0/23
15	N	SO_2NH_2	3	3.8/23

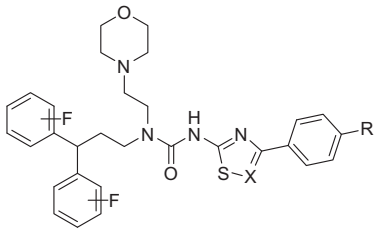
[a] Values are an average of at least two determinations.
[b] Pharmacokinetic studies were conducted in male Sprague–Dawley rats and were administered at 0.5 mg/kg IV and 2 mg/kg PO.

one kidney and 2/3 of the second kidney have been surgically removed, showed promise demonstrating a modest reduction in serum PTH levels. Despite these positive features, **1** exhibited substantial liabilities including poor pharmacokinetics (PK). This account describes our systematic efforts to address the metabolism of **1** (Fig. 1).

To understand the high clearance of **1**, a series of metabolite ID studies was performed. Incubation of **1** in the presence of rat liver microsomes (RLM) indicated sites of oxidation at the thiazole, morpholine, and *gem*-diphenyl moieties. Further studies to elaborate the specific nature of the oxidations were performed by incubating **1** in RLM containing *N*-acetylcysteine (Fig. 2). These studies suggested the intermediacy of a reactive epoxide in the formation of metabolite **1a** and implicated oxidation of the phenyl ring to a quinone methide in the formation of metabolite **1b**. The intermediacy of specific oxidations in the formation of metabolites **1a** and **1b** suggested a potential strategy to improve their stability.

Numerous reports indicate thiazoles as elements of oxidative susceptibility.[9] Our strategy to prevent epoxidation was to replace the thiazole nucleus with other heterocycles lacking the potential for epoxidation (Table 1). Despite considerable effort, only 1,2,4-thiadiazole **6** (hCaSR EC_{50} = 23 nM)[10] was identified as a competent bioisostere of **1**.

Table 4
Stabilization of the diphenyl motif by fluorination



Compound	F	X	R	hCaSR EC_{50} (nM)[a]	PK Data[b] CL (L/h/kg)/%F
16	2-F	CCl	SO_2NH_2	4	2.3/20
17	3-F	CCl	SO_2NH_2	19	—
18	4-F	CCl	SO_2NH_2	10	0.32/36
19	4-F	CCl	$NHSO_2Me$	9	0.81/1.0
20	4-F	N	SO_2NH_2	9	1.6/39
21	4-F	N	$NHSO_2Me$	20	1.3/48

[a] Values are an average of at least two determinations.
[b] Pharmacokinetic studies were conducted in male Sprague–Dawley rats and were administered at 0.5 mg/kg IV and 2 mg/kg PO.

Table 5
PK summary for compound **18**

Species	CL (L/h/kg)	Vss (L/kg)	MRT IV (h)	$t_{1/2}$ (h)	%F
Rat	0.32	2.0	6.3	7.0	36
Nonhuman Primate	0.024	0.21	8.9	8.0	15
Beagle Dog	0.098	1.2	13	11	47

An alternate strategy to reduce the oxidative susceptibility of the thiazole in **1** was to modulate reactivity by substitution at the 5-position of the thiazole. We measured time-dependent inactivation (TDI) of CYP3A4[11] as an estimate of reactive metabolite formation. Using **7**[8] as a baseline, substitution with –F, $–SO_2Me$, –CN, and –Cl was explored (Table 2 and **8**–**11**). Reduced TDI potential was observed upon substitution with chloro analog **11** proving most effective. Fluorine did not significantly decrease bioactivation of the thiazole ring (**7** vs **8**).

The chloro substituted thiazole analogue **12**[8] demonstrated a nearly 3-fold improvement in clearance relative to **1** (Table 3). Interestingly, the reverse sulfonamide analogue **13**[8] resulted in a further reduction in clearance. Unfortunately, both thiadiazole[12] analogues **14** and **15** were rapidly cleared in vivo. Further improvements to PK were realized by modification of the *gem*-diphenyl group.

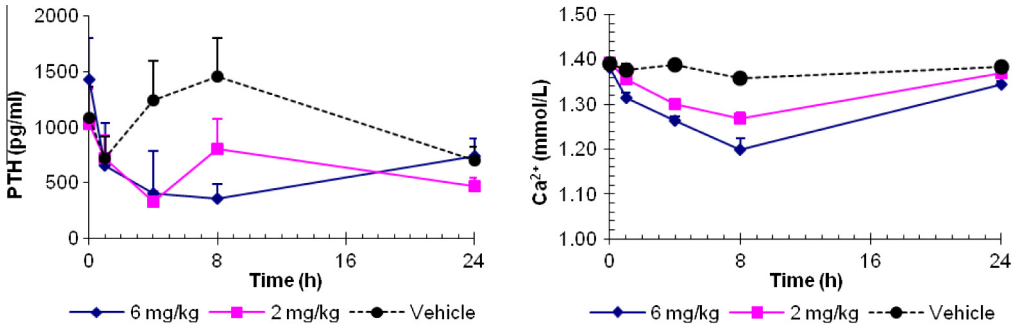
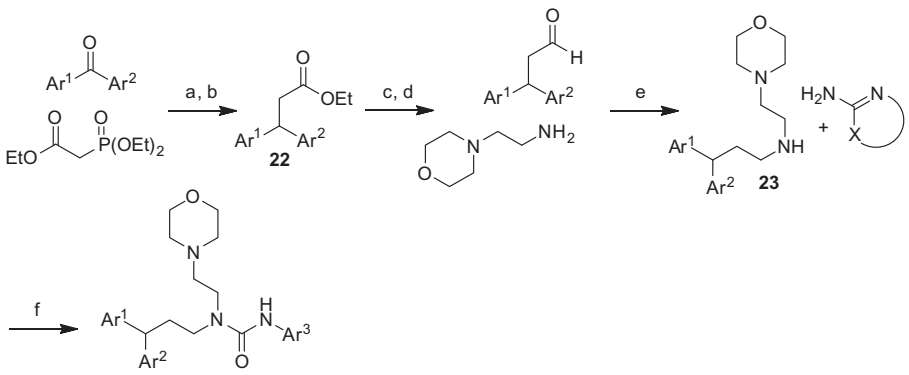


Figure 3. Sustained reduction of PTH and ionized calcium after oral administration of **18** to 5/6 nephrectomized rats.



Scheme 1. (a) NaH, PhMe, 0 °C for 1 h, then 50 °C, 3 d; (b) Pd/C, 1 atm H_2, EtOH, 25 °C, 16 h; (c) $LiAlH_4$, THF/PhMe, –78 °C to 0 °C; (d) SO_3·pyridine, Et_3N, DMSO, 25 °C; (e) $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, 25 °C; (f) Ar^3NH_2, DMAP, CDI, 40 °C, 2.5 d.

Metabolite ID studies with *N*-acetylcysteine had indicated the formation of covalent adducts at the phenyl rings suggesting the intermediacy of a quinone methide (Fig. 2, **1b**). As with the thiazole, the installation of electron withdrawing groups on the unsubstituted phenyl ring was hypothesized to reduce metabolism. Efforts focused on the incorporation of electron withdrawing elements. As such, a series of fluorinated derivatives were prepared (Table 4 and **16**–**21**). *Ortho*-fluorination resulted in a 2-fold decrease in potency relative to **13**. *Meta*- and *para*-fluorination compromised activity by 10- and 5-fold, respectively, relative to **13**. Unfortunately, the *ortho*-fluorinated derivative **16** was cleared more rapidly in rats than the parent analogue **13**.[13] In all cases, *para*-fluorination had lower clearance than their unsubstituted congeners, especially in the case of the thiadiazoles **20** and **21**.

Additional pharmacokinetic profiling of **18** in higher species confirmed the good cross-species pharmacokinetics (Table 5). These results demonstrate the pharmacokinetic improvements realized by a metabolism-guided approach to analogue design. A screen of off-target activities using a functional GPCR platform at CEREP indicated that **18** showed no confounding off-target activity.[14] Moreover, **18** exhibited robust lowering of PTH and ionized calcium (Ca^{2+}), relative to vehicle, in a 5/6 nephrectomized rat model of kidney failure when dosed orally at 6 mg/kg (Fig. 3).[15]

The modular route by which analogues were prepared is described in Scheme 1. The *gem*-diphenyl ester **22** formed from the corresponding benzophenone via a Horner–Wadsworth–Emmons reaction. Amine **23** was formed in three steps from **22** by a reduction–oxidation-reductive amination sequence. The urea functionality was formed by coupling $ArNH_2$ with carbonyldiimidazole (CDI) in the presence of 4-dimethylaminopyridine (DMAP).

In summary, we identified **18**, a novel calcimimetic with the potential to lower elevated parathyroid hormone levels associated with secondary hyperparathyroidism. A metabolism guided approach to analogue design, highlighted by the use of metabolite ID experiments and measurement of TDI of CYP3A4, addressed PK issues and enabled the identification of **18**. Most consequential was the use of thiazole chlorination in combination with fluorination of the *gem*-diphenyl moiety. Lastly, 1,2,4-thiadiazoles appear to be equipotent replacements of the chlorothiazole although the chlorothiazoles had lower rat IV clearance.

Acknowledgments

We acknowledge Roger Zanon and Yiping Wu for their assistance with formulations and rat pharmacokinetics, respectively.

References and notes

1. (a) Cunningham, J.; Locatelli, F.; Rodriguez, M. *Clin. J. Am. Soc. Nephrol.* **2011**, *6*, 913; (b) Rodriguez, M.; Nemeth, E.; Martin, D. *Am. J. Physiol. Renal Physiol.* **2005**, *288*, F253; (c) Skoreckim, K.; Green, J.; Brenner, B. M. In *Harrison's Principles of Internal Medicine*; Kasper, D. L., Braunwald, E., Fauci, A. S., Hauser, S. L., Longo, D. L., Jameson, J. L. Eda., Eds., 16th ed.; ; McGraw-Hill: New York, NY, 2005; Vol. 261, pp 1653–1663.
2. Brown, E. M.; Gamba, G.; Riccardi, D.; Lombardi, M.; Butters, R.; Kifor, O.; Sun, A.; Hediger, M. A.; Lytton, J.; Hebert, S. C. *Nature* **1993**, *366*, 575.
3. (a) Ward, B. K.; Magno, A. L.; Walsh, J. P.; Ratajczak, T. *Clin. Biochem.* **2012**, *45*, 943; (b) Nemeth, E. F. In *Principles of Bone Biology*; Bilezikian, J. P., Raisz, L. G., Rodan, G. A., Eds., 2nd ed.; Academic Press: San Diego, CA, 2002; pp 1339–1359.
4. (a) Nemeth, E. F.; Shoback, D. *Best Pract. Res. Clin. Endocrinol. Metab.* **2013**, *27*, 373; (b) Conn, P. J.; Christopoulos, A.; Lindsley, C. W. *Nat. Rev. Drug Discov.* **2009**, *8*, 41; (c) Harrington, P. E.; Fotsch, C. *Curr. Med. Chem.* **2007**, *14*, 3027; (d) Sorbera, L. A.; Castaner, R. M.; Bayes, M. *Drugs of the Future* **2002**, *27*, 831.
5. Nemeth, E. F.; Steffey, M. E.; Hammerland, L. G.; Hung, B. C. P.; Van Wagenen, B. C.; DelMar, E. G.; Balandrin, M. F. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 4040.
6. Kiefer, L.; Leiris, S.; Dodd, R. H. *Expert Opin. Ther. Patents* **2011**, *21*, 681.
7. Deprez, P.; Temal, T.; Jary, H.; Auberval, M.; Lively, S.; Guédin, D.; Vevert, J. P. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2455.
8. The discovery and optimization of **1** will be described in a future publication.
9. (a) Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, J. P. *Chem. Res. Toxicol.* **2002**, *15*, 269; (b) St. Jean, D. J.; Fotsch, C. J. *Med. Chem.* **2012**, *55*, 6002; For a similar strategy that successfully addressed the metabolic liabilities of a thiazole, see: (c) Kalgutkar, A. S.; Driscoll, J.; Zhao, S. X.; Walker, G. S.; Shephard, R. M.; Soglia, J. R.; Atherton, J.; Yu, L.; Mutlib, A. E.; Munchhof, M. J.; Reiter, L. A.; Jones, C. S.; Doty, J. L.; Trevena, K. A.; Shaffer, C. L.; Ripp, S. L. *Chem. Res. Toxicol.* **2007**, *20*, 1954.
10. The hCaSR assay was performed in human embryonic kidney 293 cells expressing the human parathyroid CaSR as previously described, see: Nemeth, E. F.; Heaton, W. H.; Miller, M.; Fox, J.; Balandrin, M. F.; Van Wagenen, B. C.; Colloton, M.; Karbon, W.; Scherrer, J.; Shatzen, E.; Rishton, G.; Scully, S.; Qi, M.; Harris, R.; Lacey, D.; Martin, D. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 627.
11. Wong, S. G.; Fan, P. W.; Subramanian, R.; Tonn, G. R.; Henne, K. R.; Johnson, M. G.; Tadano Lohr, M.; Wong, B. K. *Drug Metab. Dispos.* **2010**, *38*, 841.
12. For the preparation of substituted thiadiazoles, see: Wehn, P. M.; Harrington, P. E.; Eksterowicz, J. E. *Org. Lett.* **2009**, *11*, 5666.
13. A similar finding has been made at Pfizer using a systematic and pairwise analysis of aromatic halogenation on microsomal clearance. Sun, H.; Keefer, C. E.; Scott, D. O. *Drug Metab. Lett.* **2011**, *5*, 232.
14. Compound **18** was tested in a CEREP panel of 34 enzymes, 19 receptors, 5 transporters, and 5 channels at 10 μM. Three assays with POC < 50 (adenosine transporter, guanylyl cyclase, and carbonic anhydrase II) were not expected to interfere with the observed in vivo pharmacology. Solubility data for **18**: 0.01 N HCl = 0.017 mg/mL, Simulated Intestinal Fluid (SIF) = 0.040 mg/mL, Phosphate Buffered Saline (PBS) = 0.001 mg/mL.
15. Compound **18** was dosed orally to 5/6 nephrectomized male Sprague–Dawley rats as a suspension in 2% HPMC/1% Pluronic F68/5% Captisol in water. A statistically significant (repeated measures ANOVA, $p < 0.05$) decrease in PTH relative to vehicle was measured at 4 h for both doses and at 8 h for the 6 mg/kg dose. The decrease in blood ionized calcium was statistically significant (repeated measures ANOVA, $p < 0.05$) relative to vehicle at 1, 4, and 8 h for the 6 mg/kg dose and 4 and 8 h for the 2 mg/kg dose.