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

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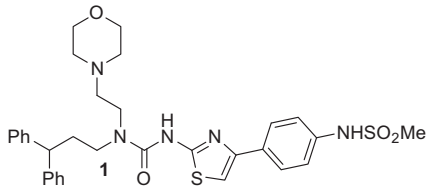
ABSTRACT

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.

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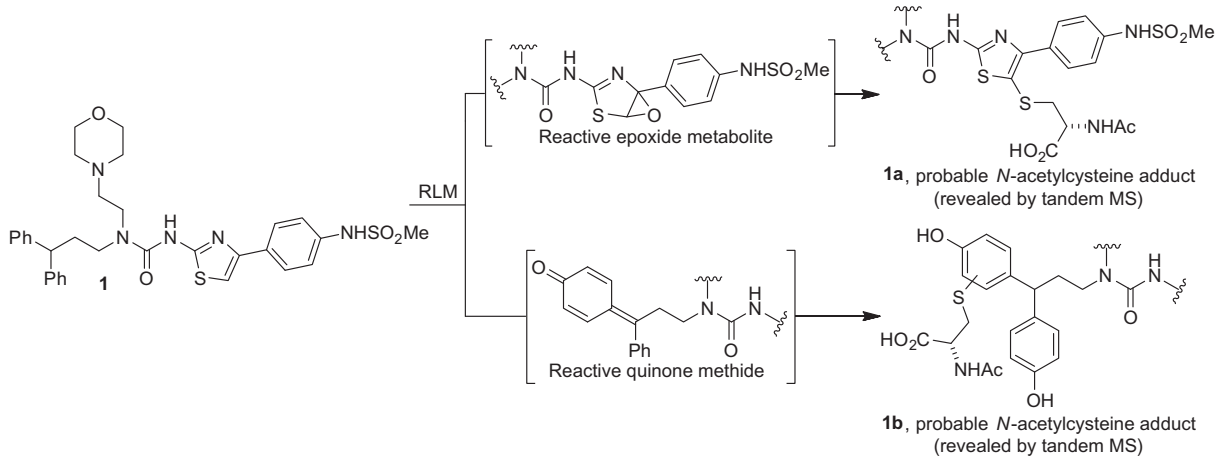
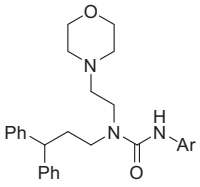
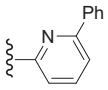
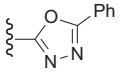
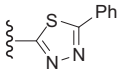
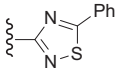
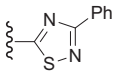


Figure 2. Metabolites of **1** formed from incubation with rat liver microsomes (RLM) and elucidated by tandem mass spectrometry.

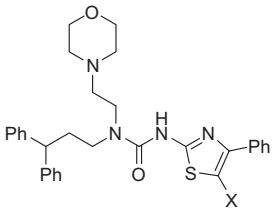
Table 1
Heterocyclic replacements of thiazole



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.

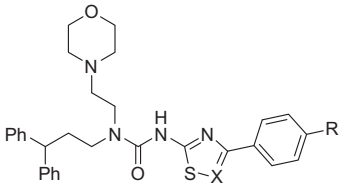
Table 2
Impact of electron withdrawing groups on TDI potential



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**.