Bioorganic & Medicinal Chemistry Letters 20 (2010) 5918–5921

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of a class of calcium sensing receptor positive allosteric modulators; 1-(benzothiazol-2-yl)-1-phenylethanols

Magnus Gustafsson [a], Jacob Jensen [a], Sine M. Bertozzi [a], Erika A. Currier [b], Jian-Nong Ma [b], Ethan S. Burstein [b], Roger Olsson [a,*]

[a] ACADIA Pharmaceuticals AB, Medeon Science Park, Per Albin Hanssons väg 35, S-205 12 Malmö, Sweden
[b] ACADIA Pharmaceuticals Inc., 3911 Sorrento Valley Blvd., San Diego, CA 92121, USA

ARTICLE INFO

Article history:
Received 20 June 2010
Revised 16 July 2010
Accepted 18 July 2010
Available online 23 July 2010

Keywords:
SAR
GPCR
CaSR
PAM
Allosteric
Modulators
Structure–activity relationship
Calciumsensing receptor
Cinacalcet
Calindol
Hyperparathyroidism
Fendiline
In vivo
Parathyroid hormone
1-(benzothiazol-2-yl)-1-phenylethanols

ABSTRACT

1-(Benzothiazol-2-yl)-1-(4-chlorophenyl)ethanol (**1**) was identified as a positive allosteric modulator (PAM) of the CaSR in a functional cell-based assay. This compound belongs to a class of compounds that is structurally distinct from other known positive allosteric modulators, for example, the phenylalkylamines cinacalcet, a modified analog (**13**) potently suppressed parathyroid hormone (PTH) release in rats, consistent with its profile as a PAM of CaSRs.

The ability of a drug to produce its effect on an organism is dependent on its molecular properties. Hence, structurally different ligands exerting their effect at the same target could potentially give rise to a ligand-specific pharmacology based on differences in pharmacokinetic properties, pharmacodynamic properties, or both.[1,2] Herein we report on the discovery and structure–activity relationship (SAR) of a novel class of calcium sensing receptor (CaSR) positive allosteric modulators (PAMs) (e.g., **1**, Fig. 1). This class of compounds is structurally distinct from the reported CaSR PAMs, for example, cinacalcet hydrochloride (**2**) and calindol.

Cinacalcet hydrochloride is the first GPCR PAM to reach the market and is used for the treatment of secondary hyperparathyroidism (SHPT) in patients with end-stage renal disease receiving hemo-dialysis and of primary hyperparathyroidism (PHPT) caused by parathyroid cancer. The essential role of the CaSR in calcium homeostasis makes it an important drug target and offers an attractive means to control hormonal disorders related to calcium homeostasis.[3,4] In addition to PHPT and SHPT, PAMs targeting the CaSR may also be effective drugs for treating mineral and bone

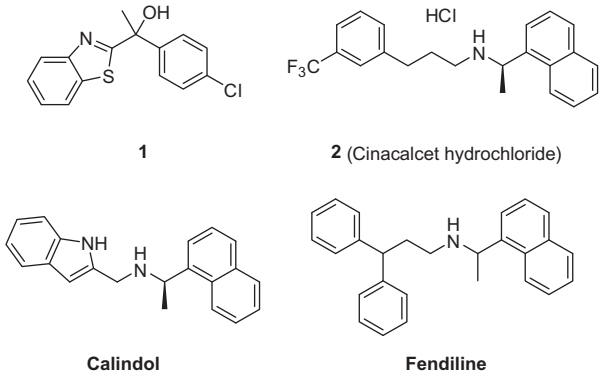


Figure 1. Selected CaSR ligands.

* Corresponding author. Tel.: +46 768874217.
E-mail address: roger@acadia-pharm.com (R. Olsson).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.077

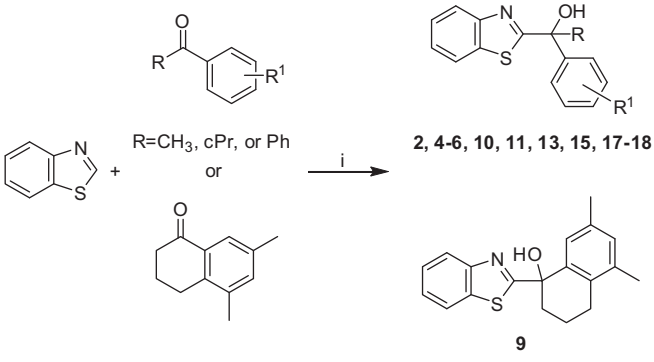
disorders in chronic kidney disease, dominant hyperparathyroidism, familial hypocalciuric hypercalcemia, neonatal severe primary hyperparathyroidism, and hypercalcemia caused by autoantibodies that inhibit CaSR.

The calcium sensing receptor is a member of the GPCR superfamily and belongs to the group C family of GPCRs and is most closely related to the $GABA_B$ receptor and metabotropic glutamate receptors.[5–8] The receptor is expressed in calciotropic hormone-secreting organs (parathyroid glands and C-cells of the thyroid glands), kidney, bone, and intestinal cells.[4] The CaSRs primarily functions are to maintain systemic Ca^{2+} homeostasis mainly by causing suppression of parathyroid hormone secretion by the parathyroid glands, and by influencing rates of renal tubular calcium reabsorption and secretion of calcitonin by C-cells of the thyroid.[9,10]

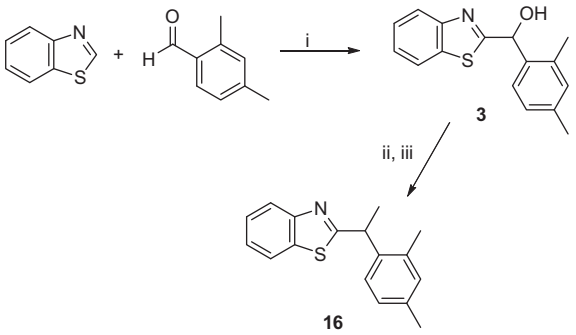
A chemical library containing 250,000 small drug-like compounds was screened for agonist activity using a cell-based functional assay called Receptor Selection and Amplification Technology (R-SAT).[11] In the screen cells were maintained in standard tissue culture medium containing Dulbecco's modified Eagle's medium (DMEM) and 0.8 mM $MgCl_2$ and 1.6 mM $CaCl_2$ to increase the sensitivity for detecting hits. A number of active compounds representing distinct structural classes were identified. Based on the structurally interesting features compared with reported PAMS, the benzothiazole class (e.g., **1**) was chosen for further studies. In contrast to **1**, most of the reported CaSR PAMs today including, NPS R-467, NPS R-568, calindol, and cinacalcet hydrochloride (**2**) are phenylalkylamines, which are structurally derived from fendiline. In addition, compound **1** is not cationic at physiological pH which further differentiates it from previously reported calcimimetics, including the PAMs, the L-amino acids, and obviously cations such as Ca^{2+} and Mg^{2+}.

Compound **1** displayed partial agonism and nM activity at the CaSR (pEC_{50} 6.4 and 68% efficacy), but also had a high intrinsic clearance in human liver microsomes of >200 μL/min mg.

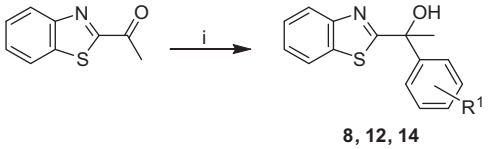
Based on the lead compound **1**, a focused set of analogues were synthesized to establish the SAR and to improve on the metabolic stability. To be able to easily introduce a variety of substituents (R) on the benzylic position, different synthetic strategies were developed (Schemes 1–4). Lithiation of benzothiazole at low temperature (−78 °C),[12] followed by addition of a variety of ketones gave products **1**, **4**–**6**, **9**–**11**, **13**, **15**, **17**–**18** in medium to good yields (Scheme 1).[13,14] Of these only the synthesis of **9** was difficult, and a modest 32% yield was achieved. The low yield was attributed to the decreased reactivity of cyclic ketone compared to the acetophenones in combination with the low stability of the lithiated benzothiazole. The lithiated benzothiazole is reported to start decomposing when increasing the temperature for at −40 °C being totally cracked.[12] To investigate the significance of the hydroxyl and the methyl group on the benzylic carbon, analogues **3** and



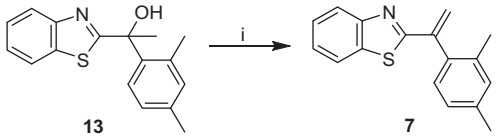
Scheme 1. Reagents and conditions: (i) *n*-BuLi, THF, −78 °C, 32–80%.



Scheme 2. Reagents and conditions: (i) *n*-BuLi, THF, −78 °C, 46%; (ii) CH_3SO_2Cl/Et_3N, CH_2Cl_2, rt; (iii) CH_3MgBr, THF, rt, 77% two steps.



Scheme 3. Reagents and conditions: (i) ArMgX, X = Cl, Br, THF, rt, 62–77%.



Scheme 4. Reagents and conditions: (i) PPA, THF/Ac_2O, rt, 41%.

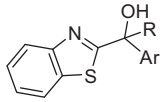
16 were synthesized. Lithiation of benzothiazole followed by reaction with 2,4-dimethylbenzaldehyde gave alcohol **3** in 46% yield (Scheme 2). Analogue **16** was made in two steps, one-pot, from alcohol **3** as follows; compound **3** was reacted with CH_3SO_2Cl/Et_3N followed by nucleophilic substitution with CH_3MgBr to yield compound **16** in 77% yield.

Analogues **8**, **12**, and **14** were made by reacting acetylbenzothiazole with the corresponding Grignard reagent (Scheme 3). Treatment of compound **13** with poly phosphoric acid (PPA) gave exo-methylene **7** in 41% yield (Scheme 4).

The in vitro activity of these analogs was measured using R-SAT (Table 1). Experiments using media partially depleted of $MgCl_2$ and various concentrations of $CaCl_2$ confirmed that our compounds cooperate with divalent cations to activate the CaSR (Fig. 2).[15]

From the SAR it reads that the phenyl substituents have a large impact on the activity of this class of compounds (Table 1). Replacing the phenyl with the larger naphthyl gives a slightly less active analogue compared with the hit **1**. A more rigid structure was well tolerated, and analogue **9** retained the activity (pEC_{50} 6.5 ± 0.2 and Eff. 81 ± 6). Whereas the *o*-methyl compound **11** had comparable activity with the *p*-chloro compound **1**, the electron donating *o*-methoxy group (**10**) had a negative effect and the activity dropped 10 times to pEC_{50} 5.4. With these substantial changes in activity in mind we further elaborated with small substituents, for example, Me, Cl, and F to give an initial SAR. Compounds **11** (*o*-CH_3) and **12** (*m*-CH_3-*p*-F), pEC_{50} at 6.6 and 6.8, respectively, had slightly higher activity than the hit **1** (pEC_{50} 6.4). However, a more substantial increase in activity was seen when a disubstitution pattern with methyl and chloro groups was introduced (Table 1, compounds **5** and **13**–**15**). The two most active racemic compounds tested were **13** (pEC_{50} 7.6 ± 0.1 Eff. 91 ± 4) and **14** (pEC_{50} 7.5 ± 0.2 Eff. 82 ± 9). Another predominant feature of the structure–activity relationship is that a small non-hydrogen R substituent is manda-

Table 1
In vitro activities of CaSR agonists using R-SAT[11]



Compd	R	Ar	pEC_{50}	%Eff.[a]
2	Cinacalcet hydrochloride		7.7(±0.4)	115 (±6)
1	Me	*p*-ClPh	6.4 (±0.1)	68 (±7)
3	H	*o*,*p*-di-MePh	na	na
4	Ph	*m*,*p*-di-ClPh	na	na
5	Me	*m*,*p*-di-ClPh	7.0 (±0.1)	62 (±7)
6	*c*Pr	*o*,*p*-di-MePh	6.7 (±0.1)	76 (±6)
7			na	na
8	Me	β-naphthyl	6.2 (±0.1)	53 (±7)
9			6.5 (±0.2)	81 (±6)
10	Me	*o*-MeOPh	5.4 (±0.1)	26 (±6)
11	Me	*o*-MePh	6.6 (±0.1)	72 (±7)
12	Me	*p*-F,*m*-MePh	6.8 (±0.1)	62 (±7)
13	Me	*o*,*p*-di-MePh	7.6 (±0.1)	91 (±4)
(*S*)-**13**			8.0 (±0.1)	83 (±5)
(*R*)-**13**			7.1 (±0.1)	79 (±4)
14	Me	*p*-Cl,*o*-MePh	7.5 (±0.2)	94 (±4)
15	Me	*o*,*p*-di-ClPh	7.0 (±0.1)	82 (±9)
16			5.6 (±0.3)	88 (±12)

[a] Values are means of at least three independent experiments, standard deviation is given in parentheses (na—not active at 10 nM).

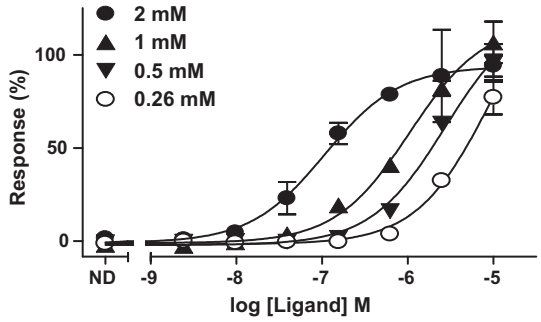
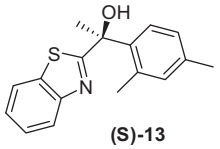


Figure 2. In vitro assay of compound **13** in DMEM with 0.5 mM $MgCl_2$ and indicated $CaCl_2$ concentrations.

tory for activity (Table 1). If R is a hydrogen atom no activity was observed (Table 1, compound **3**). Changing R from methyl to cyclopropyl reduces activity approximately 10-fold (see Table 1, compare compound **13** to compound **6**). Moreover, a somewhat larger R, that is, phenyl, (**4**) abolishes activity at the CaSR. In order to examine the influence of the hydroxyl group on activity compound **16** was tested. This analogue (**16**) turned out to be 100-fold less active compared to the hydroxyl containing analogue (**13**) in our assays, indicating the importance of the hydroxyl group for this series of compounds. In addition, one potential structural liability for this structure class is the possible elimination of the tertiary alcohol by formation of an exo-methylene under the assay conditions used. However, to rule this out compound **7** was tested and found to be inactive. Therefore, exo-methylene derivatives are not responsible for the activity seen in this series of compounds.

The enantiomers of **13** were separated using chiral HPLC (ee >99%) and each enantiomer was tested for agonist activity at CaSR. It was found that the *S*-enantiomer (*S*)-**13** (pEC_{50} 8.0 ± 0.1 and Eff. 83 ± 5, Fig. 3) was approximately 10-fold more active than the *R*-enantiomer (*R*)-**13** (pEC_{50} 7.1 ± 0.1 and %Eff. 79 ± 4). The R-SAT activities and rank order potencies of compounds **13**, (*R*)-**13**, and (*S*)-**13** were further confirmed in phosphatidyl inositol (PI) hydrolysis assays. Compound **13** did not activate human $GABA_B$ recep-



(S)-13

pEC_{50} 8.0 ± 0.1, %Eff. 83 ± 5

Figure 3. Absolute configuration and in vitro activity of (*S*)-**13**.

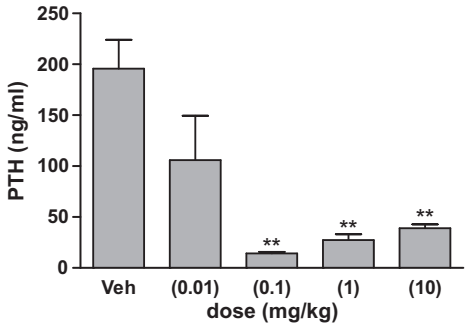


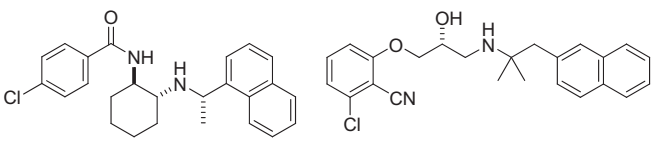
Figure 4. In vivo activity of compound **13**. The indicated doses of compound **13** were administered to male Sprague–Dawley rats subcutaneously, blood samples taken at 1 h post-dose, and plasma PTH levels measured using ELISA. ** p <0.01.

tors, or human type 1 parathyroid hormone (PTH1) receptors, confirming its specificity for the CaSR, and potently suppressed parathyroid hormone (PTH) release in rats (Fig. 4), consistent with its profile as a PAM of CaSRs.[15]

The clearance in human liver microsomes of **13** is 396 μL/min mg, which is not an improvement compared with the initial lead **1** (>200 μL/min mg). However, the clearance was reduced by exchanging the *ortho*- and *para*-methyl substituents on the phenyl to chloro substituents (**15**). The latter compound had a clearance of 66 μL/min mg which represents a moderately cleared compound in human liver microsomes.

Calindol and NPS R-568 (the latter a close analogue of cinacalcet) have been suggested, using receptor mutation studies, to be primarily anchored through a strong H-bond salt bridge between the basic amine in the compounds and a glutamate (E837) in the CaSR.[16] A strong H-bond salt bridge is not likely with the series presented herein; the calculated pK_a is 1.8 for **13**. However, in docking studies the calcilytics NPS 2143 and Calhex 231 (Fig. 5) have been suggested to form hydrogen bonds to E837 in addition to a salt bridge between the basic nitrogen and glutamate. This binding mode might be possible with for example compound **1**, as it was shown in the SAR that the alcohol was important for activity compare compounds **13** and **16**. The OH could potentially support a weak H-bond salt bridge. Consistent with this idea, we observed that the E837A mutation reduced the activity of cinacalcet more than the activity of **13**[15].

In conclusion we have described the discovery, SAR, and characterization of a new class of PAMs which is structurally distinct



Calhex 231 NPS 2143

Figure 5. Calcilytics.

from the currently known compounds. Their main structural feature is the absence of a basic nitrogen and that they are neutral at physiological pH.

Acknowledgment

We thank Associate Professor Lars Eriksson for determining the X-ray structure of compound (*S*)-**13**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.077.

References and notes

1. Lewis, J. A.; Lebois, E. P.; Lindsley, C. W. *Curr. Opin. Chem. Biol.* **2008**, *12*, 269.
2. Thomas, R. L.; Mistry, R.; Langmead, C. J.; Wood, M. D.; Challis, R. A. J. *J. Pharmacol. Exp. Ther.* **2008**, *327*, 365.
3. Trivedi, R.; Mithal, A.; Chattopadhyay, N. *Curr. Med. Chem.* **2008**, *15*, 178. and references cited therein.
4. Jensen, A. A.; Bräuner-Osborne, H. *Curr. Neuropharmacol.* **2007**, *5*, 180.
5. Brown, E. M.; MacLeod, R. J. *Physiol. Rev.* **2001**, *81*, 239. and references cited therein.
6. Hofer, A. M.; Brown, E. M. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 530.
7. Bettler, B.; Kaupmann, K.; Mosbacher, J.; Gassmann, M. *Physiol. Rev.* **2004**, *84*, 835.
8. Jensen, A. A. In *Molecular Neuropharmacology. Strategies and Methods*; Schousboe, A., Bräuner-Osborne, H., Eds.; Humana Press, 2004; pp 47–82.
9. Chattopadhyay, N.; Mithal, A.; Brown, E. M. *Endocr. Rev.* **1996**, *17*, 289.
10. Brown, E. M. *Nat. Clin. Pract. Endocrinol. Metab.* **2007**, *3*, 122.
11. (a) Burstein, E. S.; Piu, F.; Ma, J. N.; Weissman, J. T.; Currier, E. A.; Nash, N. R.; Weiner, D. M.; Spalding, T. A.; Schiffer, H. H.; Del Tredici, A. L.; Brann, M. R. *Curr. Pharm. Des.* **2006**, *12*, 1717; (b) Lund, B. W.; Piu, F.; Gauthier, N. K.; Eeg, A.; Currier, E.; Sherbukhin, V.; Brann, M. R.; Hacksell, U.; Olsson, R. *J. Med. Chem.* **2005**, *48*, 7517; (c) Piu, F.; Gauthier, N. K.; Olsson, R.; Currier, E. A.; Lund, B. W.; Croston, G. E.; Hacksell, U.; Brann, M. R. *Biochem. Pharmacol.* **2005**, *71*, 156; (d) Seitzberg, J. G.; Knapp, A. E.; Lund, B. W.; Bertozzi, S. M.; Currier, E. A.; Ma, J.-N.; Sherbukhin, V.; Burstein, E. S.; Olsson, R. *J. Med. Chem.* **2008**, *51*, 5490; (e) Lund, B. W.; Eeg, A.; Piu, F.; Gauthier, N. K.; Begtrup, M.; Hacksell, U.; Olsson, R. *J. Med. Chem.* **2009**, *52*, 1540; (f) Pettersson, H.; Bülow, A.; Ek, F.; Jensen, J.; Ottesen, L. K.; Fejzic, A.; Ma, J.-N.; Del Tredeci, A. L.; Currier, E. A.; Gardell, L. R.; Tabatabaei, A.; Craig, D.; McFarland, K.; Ott, T. R.; Piu, F.; Burstein, E. S.; Olsson, R. *J. Med. Chem.* **2009**, *52*, 1975; (g) Gaubert, G.; Bertozzi, F.; Kelly, N. M.; Pawlas, J.; Scully, A. L.; Nash, N. R.; Gardell, L. R.; Lameh, J.; Olsson, R. *J. Med. Chem.* **2009**, *52*, 6511.
12. The reagent 2-benzothiazolyllithium has been reported unstable above −35 °C, see Refs. 13 and 14.
13. Gilman, H.; Beel, J. A. *J. Am. Chem. Soc.* **1949**, *71*, 2328.
14. Chikashita, H.; Ishibaba, M.; Ori, K.; Itoh, K. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 3637.
15. Manuscript in preparation.
16. Petrel, C.; Kessler, A.; Dauban, P.; Dodd, R. H.; Rognan, D.; Ruat, M. *J. Biol. Chem.* **2004**, *279*, 18990.