Bioorganic & Medicinal Chemistry Letters 20 (2010) 5161–5164

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Penta-substituted benzimidazoles as potent antagonists of the calcium-sensing receptor (CaSR-antagonists)

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ARTICLE INFO

Article history:
Received 9 June 2010
Revised 2 July 2010
Accepted 3 July 2010
Available online 3 August 2010

Keywords:
Calcium-sensing receptor (CaSR)
Parathyroid hormone (PTH)
Bone formation
Osteoporosis
CaSR-antagonist
Benzimidazoles

ABSTRACT

A series of novel benzimidazole derivatives has been designed via a scaffold morphing approach based on known calcilytics chemotypes. Subsequent lead optimisation led to the discovery of penta-substituted benzimidazoles that exhibit attractive in vitro and in vivo calcium-sensing receptor (CaSR) inhibitory profiles. In addition, synthesis and structure–activity relationship data are provided.

Osteoporosis is a disease characterised by low bone mass and microarchitectural deterioration of bone tissue that leads to bone fragility and increased risk of fractures.[1] Most therapies for the treatment of osteoporosis inhibit bone resorption and prevent further bone loss. However, as many osteoporosis patients have already lost a substantial amount of bone at the time of diagnosis there is a need for agents that stimulate new bone formation.[2] The only currently commercially available anabolic treatment for osteoporosis are PTH (parathyroid hormone) or PTH fragments such as Forteo® (teriparatide, the 1–34 fragment of PTH), which causes a significant increase in bone mass and reduces vertebral fracture risk substantially.[3] However, this peptide must be administered by sc injections. An orally active low molecular weight compound with the same efficacy would be a highly attractive alternative for the patient. Instead of applying *exogenous* PTH, mobilisation of *endogenous* stores of the hormone can be envisaged. PTH is stored in relatively large amounts in parathyroid cells and its secretion is controlled by a calcium-sensing receptor (CaSR) located on the cell surface. As the release of PTH is *negatively* coupled to CaSR activation, CaSR-antagonists (calcilytics) mimic a state of hypocalcemia and thus stimulate PTH release into the blood stream.[4]

It is well documented that elevated levels of PTH only result in higher bone mass if they are transient, that is, they do not persist for more than about 2–4 h. Elevated PTH levels sustained for several hours activate not only osteoblasts but also osteoclasts leading to an increase in bone turnover instead of higher bone mass.[5] Therefore, the drug discovery goal of this approach is the identification of a CaSR-antagonist, that is, rapidly absorbed (short T_{max}) and fairly rapidly eliminated. At Novartis the starting point for lead optimisation towards a compound exhibiting such properties was the HTS hit **1** (Fig. 1), a close analogue of Proquazone (an analgesic). Lead optimisation led to compounds such as **2** and **3** that showed

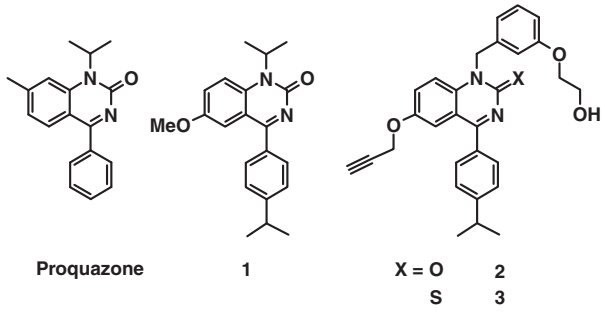

Figure 1. Proquazone and proquazone type calcilytics.

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doi:10.1016/j.bmcl.2010.07.016

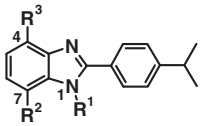
IC_{50} values in the nanomolar range in the in vitro receptor assay and also displayed the appropriate PK- and PTH-release properties as demonstrated in in vivo experiments in rats and dogs (short T_{max}, sharp PTH plasma peak).[6]

In an attempt to identify another template, three structures **4**, **5** and **6** of the proquazone (or structurally related) series were superimposed as shown in Figure 2. Based on this superimposition a new template **7**, a benzimidazole derivative, displaying the structural features thought to be necessary for adequate receptor affinity, was designed (morphed) and subsequently prepared. Testing of **7** revealed an IC_{50} of 2.5 μM in the in vitro assay.

A fluorimetric assay was used as the primary screen to determine inhibitory potency of the compounds against the CaSR. The assay (FLIPR assay) measures calcium mobilisation in hamster fibroblasts transfected with the human receptor.[6]

At the initial stages of lead optimisation (Table 1), derivatives **7–9** showed that methoxy-ethyl as the R^1 residue (leading to slightly more soluble compounds, compared to the *n*-butyl derivatives) can easily replace *n*-butyl and that the isopropyloxy residue (R^3) can be replaced by the smaller methoxy without any significant loss in potency. The unsubstituted derivative **10** (R^1, R^2 = H) was less active. It could also be demonstrated that moving the methoxy residue from the 4 to the 7 position (**11**) is tolerated. Replacement of the 7-OMe residue with a methyl-group was also tolerated (**12**). Introduction of an additional methyl residue at position 7 of **9** leading to **13** did not have much impact on potency of the resulting compound. Surprisingly, however, was the 10-fold increase in potency of **14** where the positions of the 4- and the 7-residues have been switched. Further increases in activity could be achieved with the introduction of halogens or trifluoromethyl residues at position 4, with bromo- and iodo-residues being most preferred (**15**, **16**, **17** and **18**). The 4,7-dimethoxy- and the 4-methoxy-7-chloro-derivatives **19** and **20** proved to be significantly less potent than the 4-chloro-7-methoxy derivative **15**. It was observed, that fairly lipophilic R^1 residues were needed for good potency. The *N*-dimethylamino-ethyl derivative **22** showed a ca. 10-fold lower activity in comparison with the methoxy-ethyl compound **16** and the propyl derivative **23** showed a fourfold decrease in potency. An amide moiety as present in **24** led to a substantial loss of activity.

Table 1
In vitro activity of compounds **7–24**



Compd	R^1	R^2	R^3	CaSR FLIPR IC_{50} (μM)
7	*n*-Bu	H	*i*-PrO	2.55
8	Methoxy-ethyl	H	*i*-PrO	1.95
9	*n*-Bu	H	MeO	4.69
10	Methoxy-ethyl	H	H	17
11	Methoxy-ethyl	MeO	H	1.90
12	Methoxy-ethyl	Me	H	2.10
13	Methoxy-ethyl	Me	MeO	1.90
14	Methoxy-ethyl	MeO	Me	0.21
15	Methoxy-ethyl	MeO	Cl	0.05
16	Methoxy-ethyl	MeO	Br	0.022
17	Methoxy-ethyl	MeO	I	0.013
18	Methoxy-ethyl	MeO	CF_3	0.051
19	Methoxy-ethyl	MeO	MeO	1.82
20	Methoxy-ethyl	Cl	MeO	0.77
21	Methoxy-ethyl	Br	Br	1.00
22	*N*,*N*-Dimethyl-amino-ethyl	MeO	Br	0.19
23	*n*-Pr	MeO	Me	0.79
24	*N*,*N*-Dimethyl-acetamido	MeO	Br	>10

In order to further increase the potency of these benzimidazole derivatives certain additional residues in position 5 can be beneficial as shown in Table 2. At position 5, halogens and especially a benzyl residue in combination with a halogen or trifluoro moiety at position 4 led to a slight increase in potency, while a cyano residue was just tolerated (compounds **25–29** and **33**). However, an ethyl or phenyl residue led to loss in potency (compounds **30** and **32**). The reduced activity of **31** showed that substitution of the 5 position alone is not sufficient for producing potent compounds and that a residue (e.g., halogen or trifluoromethyl) at position 4 was needed for good activity. Introduction of additional substituents in the meta- and para-positions of the 5-benzyl residues led to a decrease in activity (compounds **34–36**), while introduction of an ortho SO-Me moiety (**37**) resulted in a highly potent compound. Replacement of 5-benzyl with a 3-pyridine-methyl- (**38**) group was tolerated as well. Introduction of an additional substitutent such as 2-methoxy, 2-methyl-sufanyl and 2-methyl-sulfinyl into the pyridine moiety was not only well tolerated but can lead to a further slight increase in potency as shown specifically for compounds **39–42**. The thiazolyl-methyl-moiety (**43**) led to another highly potent compound.

The synthesis scheme (Scheme 1) illustrates the synthetic preparation of the compounds **40**, **41** and **42**. The commercially available aniline **44** was converted into **45** via O-methylation and Sandmeyer reaction. Introduction of the methoxyethyl-amino residue via nucleophilic substitution of the chlorine by treatment with methoxy-ethylamine, followed by selective bromination at position 5 led to **47** in a reasonable overall yield. Reduction of the nitro group with Raney-Ni, acylation of the aniline nitrogen with 4-isopropyl benzoic acid followed by cyclisation to the benzimidazole derivative **48** proceeded in almost quantitative overall yield. Palladium assisted replacement of the bromo moiety by a cyano residue followed by Raney-Ni reduction afforded the aldehyde **49** in moderate yield. Reaction of **49** with the lithium derivative obtained from **50**

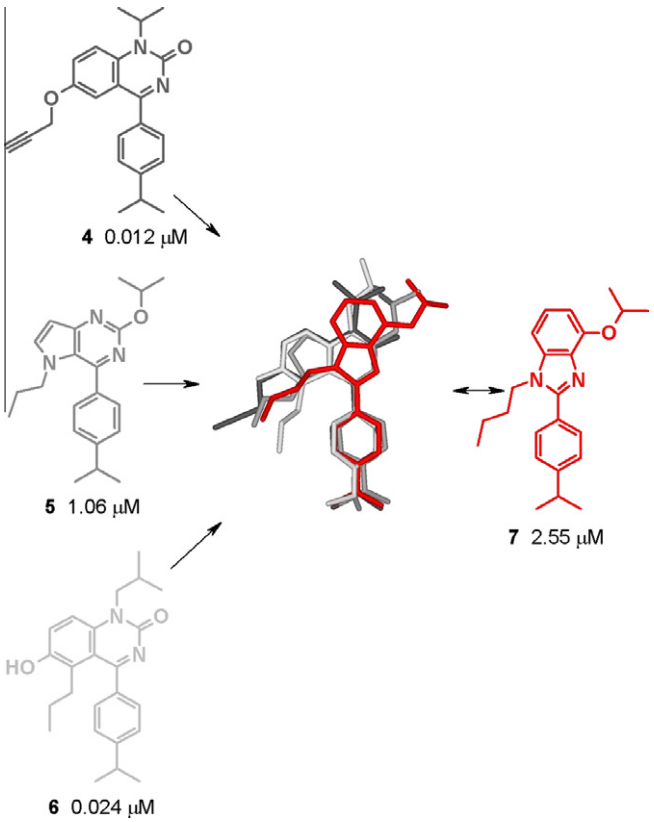
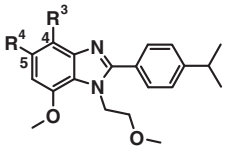
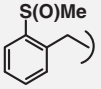
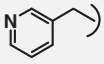
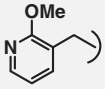
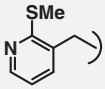
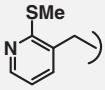
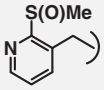


Figure 2. Overlay of structures and scaffold morphing.

Table 2
In vitro activity of compounds **25–37**



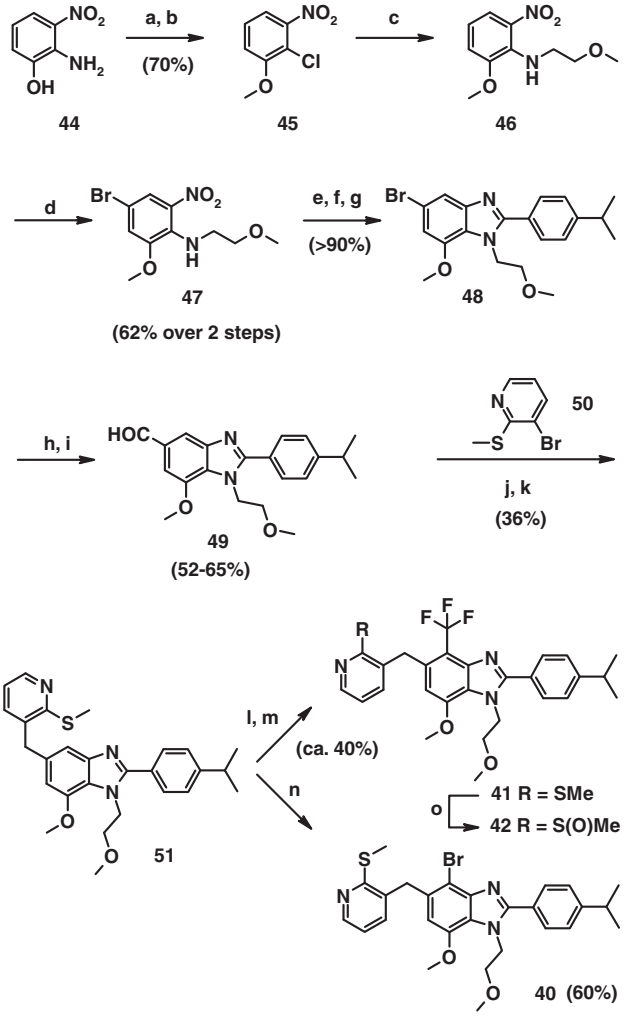
Compd	R^4	R^3	CaSR FLIPR IC_{50} (μM)
25	Br	Br	0.014
26	Br	I	0.006
27	CF_3	Br	0.011
28	Br	CF_3	0.016
29	CN	Br	0.025
30	Et	Br	0.19
31	CF_3	H	0.36
32	Ph	Br	0.06
33	Bn	Br	0.005
34	2-Methoxy-benzyl	CF_3	0.026
35	3,4-Dimethoxy-benzyl	Br	0.043
36	3,4-Dimethyl-benzyl	Br	0.34
37		Br	0.005
38		Br	0.015
39		CF_3	0.004
40		Br	0.004
41		CF_3	0.0016
42		CF_3	0.0026
43	2-Thiazolyl-2-methyl	CF_3	0.002

by Br–Li exchange with *n*-butyl-lithium and subsequent reduction of the resulting secondary alcohol led to the 5-benzyl-benzimidazole derivative **51**. Iodination of **51** at position 4, and conversion of the iodo-substituent into a trifluoromethyl residue using methyl-2,2-difluoro-2-(fluorosulfonyl) acetate[7] led to **41** in moderate yields, while bromination of **51** with bromine in acetic acid afforded **40** in better yields. Hydrogen peroxide mediated oxidation of the sulphur in **41** afforded the sulfinyl derivative **42**.

All other compounds described in this letter can be prepared starting from the appropriately substituted di-aniline derivatives as described above for the preparation of **40–42** (Scheme 1) or by using previously published synthetic procedures.[8]

As pointed out in the introductory part of the letter, a useful calcilytic compound (CaSR-antagonist) must be rapidly absorbed (short T_{max}) upon po dosing and fairly rapidly eliminated, thus inhibiting the target only for few hours per day (leading to elevated PTH levels for 2–4 h).

Based on its overall in vitro and physicochemical properties, **40** was selected for in vivo PK/PD assessment.[6] As shown in Figure 3, in dogs **40**, dosed at 3 mg/kg po (in a microemulsion formulation) reached its maximum plasma concentration of 155 nM within 2 h post administration, with a significant decrease at the 3 h time point. Six hours post dosing the plasma levels of **40** had decreased to a level of ca. 14% of C_{max}. An excellent PK/PD relationship was observed, with maximal PTH concentration/drug levels at ca. 2 h post dosing. The overall shape of the PTH curve followed nicely



Scheme 1. Preparation of **40**, **41** and **42**. Reagents and conditions: (a) NaH, MeI, DMF; (b) $NaNO_2$, CuCl, HCl; (c) 2-methoxyethylamine; Hünig's base, sealed tube, △; (d) Br_2, HOAc; (e) Raney-Ni, THF, H_2; (f) isopropylbenzoic acid, EDC, DMAP, CH_2Cl_2; (g) HOAc, 100 °C; (h) $Zn(CN)_2$, DMF, tetrakis(triphenylphosphine)palladium; (i) Raney-Ni, sodiumhypophosphite, water, pyridine, HOAc; (j) *n*-BuLi; (k) Zn, HCOOH; (l) I_2, Ag_2CO_3, AcOH, 80 °C; (m) CuI, FSO_2-CF_2COOMe, DMF, 120 °C; (n) Br_2, HOAc; (o) H_2O_2, H_2O, AcOH.

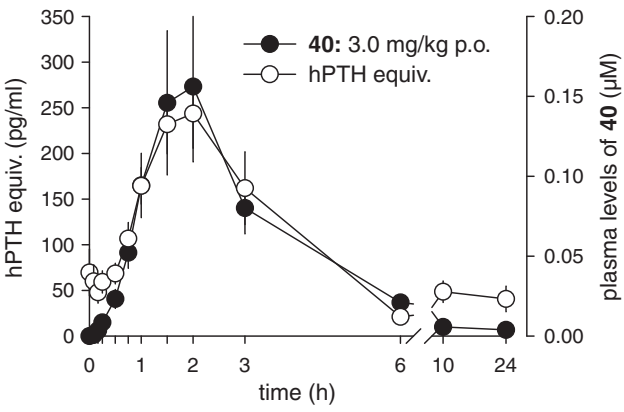


Figure 3. Plasma levels of PTH as determined by ELISA and of **40** as determined by LC/MS after oral administration of 30 mg/dog (ca. 3 mg/kg) in a microemulsion formulation.

the shape of the compound plasma concentration curve. The PTH peak levels are considered sufficient for achieving a bone anabolic effect.

In summary, a novel series of benzimidazoles has been designed and optimised as inhibitors of the calcium-sensing receptor (calcilytics). A selected example has been shown to be a potent calcilytic with in vivo activity. Compound **40** showed a sharp PTH-release peak in dogs after oral administration in a microemulsion formulation.

Acknowledgements

The authors would like to thank Esther Roehn, Karin Ryffel, Gerard Schreiber, Nina Svennum, Sandra Ammon Vetter, Jean-Louis Runser, Heinz Anklin, Valérie Cordier, Esther Scheidegger and Sven Guldbransen for excellent technical assistance.

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