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Letters

Discovery and Characterization of Novel, Potent, Non-Peptide Parathyroid Hormone-1 Receptor Antagonists

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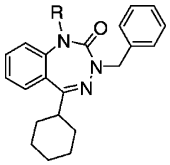
Abstract: A 1,3,4-benzotriazepine was identified as a suitable lead in our effort toward obtaining a non-peptide parathyroid hormone-1 receptor (PTH_1R) antagonist. A process of optimization afforded derivatives displaying nanomolar PTH_1R affinity, a representative example of which behaved as a PTH_1R antagonist in cell-based cyclic adenosine monophosphate (cAMP) assays, with selectivity over PTH_2 receptors.

End-stage cancer is often accompanied by hypercalcaemia. This condition, known as malignancy-associated hypercalcaemia (MAHC[a]), is caused by parathyroid hormone related peptide (PTHrP), otherwise not normally present in the circulation, being secreted by tumor cells and acting on PTH_1 receptors (PTH_1-Rs) on the kidney and/or on osteoblasts. Stimulation of this latter target results in particularly debilitating sequelae, since bone is a fertile environment for metastasized tumor cells including those from breast, lung, prostate, kidney, and multiple myeloma. Osteoblasts, once activated by tumor cell derived PTHrP, are bone forming, but their stimulation results in production of secondary signaling molecules that promote maturation of bone lytic osteoclasts. Not only does the resultant osteolysis exacerbate tumor cell metastasis but the attendant bone matrix cytokine release and local elevated Ca^{2+} also promote tumor cell proliferation in addition to stimulating further release of tumor-derived PTHrP.[1]

There is now credible evidence of a beneficial role in MAHC and in osteolytic metastasis for therapeutic interventions that block the interaction of PTHrP with its receptor. Unlike parathyroid hormone (PTH) (an 84 amino acid peptide circulating hormone produced by the parathyroid glands), which interacts with two distinct type B G-protein-coupled receptors (GPCRs), PTH_1 and PTH_2, PTHrP is a selective stimulant of PTH_1 receptors.

The hypercalcaemia and skeletal metastasis induced by inoculation of animals with PTHrP-producing cancer cells was prevented by using an anti-PTHrP antibody (Ab).[2,3] The pivotal role played by PTHrP in this setting was apparent, since the oncological phenotype was more pronounced in animals inoculated with cancer cells expressing PTHrP than in those with cancer cells that expressed little or none.[4,5] Moreover, anti-PTHrP Ab treatment did not have a significant effect on the metastasis to visceral organs, indicating the importance of the bone environment in this pathology.[6] Anti-PTHrP Ab treatment

Table 1. Biological Data for 1,3,4-Benzotriazepine-Based Compounds[a]



compd	R	PTH_1[b]
1	4-$C_6H_4NHC(=NH)NH_2$	6.15 ± 0.12
2	4-$C_6H_4NH_2$	39% (1 μM)[c]
3	4-$C_6H_4CO_2H$	<4
4	3-$C_6H_4NHC(=NH)NH_2$	5.55
5	$(CH_2)_3NHC(=NH)NH_2$	51% (30 μM)[c]
6	4-$CH_2C_6H_4NHC(=NH)NH_2$	6.03 ± 0.12

[a] Data were generally obtained from at least three separate experiments. Where no SEM is recorded, the data were obtained from two experiments. [b] $pK_I \pm$ SEM values obtained from competition with [^{125}I]-[Nle,^{8,18} Tyr^{34}]-hPTH(1−34) for recombinant $hPTH_1Rs$ in HEK293 cell membranes. [c] Where pK_I could not be determined, the percentage inhibition achieved at the given concentration is recorded.

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[a] Abbreviations: MAHC, malignancy-associated hypercalcaemia; PTHrP, parathyroid hormone related peptide; PTH_1Rs, PTH_1 receptors; PTH, parathyroid hormone; GPCRs, G-protein-coupled receptors; Ab, antibody; $hPTH_1Rs$, human PTH_1 receptors; CGRP, calcitonin gene-related peptide; CRLR, calcitonin receptor-like receptor; RAMP, receptor activating modifying protein; ADME, absorption, distribution, metabolism, and excretion; cAMP, cyclic adenosine monophosphate.

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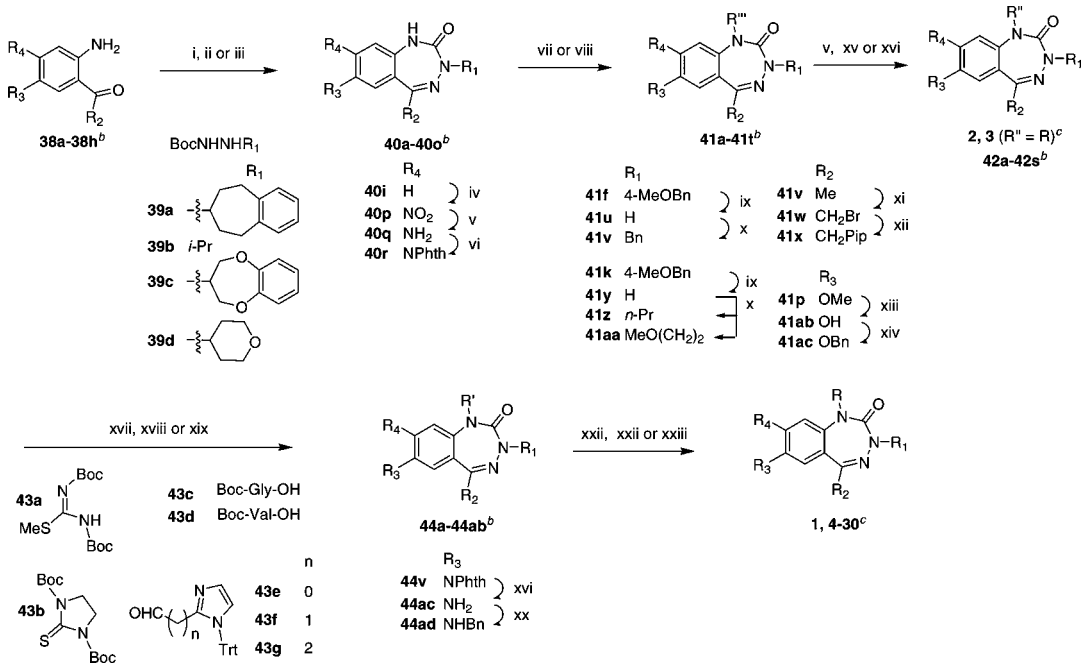
Scheme 1. Synthesis of 1,3,4-Benzotriazepines[a]



[a] Reagents and conditions: (i) (a) $(Cl_3CO)_2CO$, NEt_3, R_1NHNH_2/DCM; (b) *p*-TSA/*i*-PrOH; (ii) (a) Cl_2CS, NEt_3, R_1NHNH_2/DCM, then *p*-TSA/*i*-PrOH; (b) NaOMe, H_2O_2/MeOH−THF; (iii) (a) $(Cl_3CO)_2CO$, NEt_3, **39a**−**d**/DCM; (b) CF_3CO_2H/DCM; (iv) KNO_3, H_2SO_4; (v) $SnCl_2 \cdot H_2O$ /EtOAc; (vi) phthalic anhydride/DMF; (vii) $Cu(OAc)_2$, Cu, R‴F, K_2CO_3/DMF; (viii) NaH, R‴Br/DMF; (ix) CF_3CO_2H, Δ; (x) NaH, R_1Br/DMF; (xi) Br_2, HOAc/DCM; (xii) piperidine/DCM; (xiii) BBr_3/DCM; (xiv) K_2CO_3, BnBr/DMF; (xv) NaOH/EtOH; (xvi) $N_2H_4 \cdot H_2O$/EtOH; (xvii) **43a, 43b**, $HgCl_2$/DCM; (xviii) **43c, 43d**, EDCI, HOBt, DMAP/DMF; (xix) **43e**−**g**, $NaBH(OAc)_3$, HOAc/DCE; (xx) PhCHO, $NaBH(OAc)_3$, HOAc/DCE; (xxi) HCl/dioxane; (xxii) CF_3CO_2H, room temp; (xxiii) AcOH/MeOH. [b] See Supporting Information for definitions of R‴, R″, R″, R, R_1, R_2, R_3, R_4. [c] See Tables 1 and 2 for definitions of R, R_1, R_2, R_3, R_4.

also stimulated lean body growth and inhibited hypercalcaemia in a mouse model of lung cancer-induced cachexia.[7] There is also evidence of direct proliferative effects of PTHrP on tumor cell growth,[8,9] suggesting that PTH_1R stimulation may also contribute to the progression of certain primary tumors.

Inhibition of the effects of PTHrP has also been achieved with PTH_1R antagonists.[10] Until recently, these have been limited to peptide-based compounds derived largely from truncation of the N-terminal region of PTH,[11] PTHrP,[12] or TIP39 (a selective stimulant of PTH_2 receptors).[13,14] We considered that a small molecule, non-peptide PTH_1R antagonist, may have greater therapeutic potential. The first compound of this type has only been described recently[15] and follows the first report of a non-peptide PTH_1R agonist.[16] Herein, we describe our work in the discovery and characterization of a novel series of small-molecule, non-peptide PTH_1R antagonists.

When a selected subset of compounds from our collection were screened in a radioligand binding assay using recombinant human PTH_1 receptors ($hPTH_1Rs$) expressed in HEK293 cells, the 1,3,4-benzotriazepine-based compound **1** was found to display micromolar affinity (Table 1). Using **1** as a lead, we embarked on a program of optimization aimed at obtaining derivatives (Scheme 1) with suitable potency and physicochemical and ADME characteristics to explore in osteolytic metastasis.

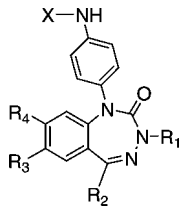
Initial derivatives of **1** (Table 1) suggested that a *p*-guanidinylphenyl N-1 substitutuent was preferred, since compounds obtained on replacement of guanidine by amino (**2**) and carboxy (**3**) were less potent. Lower affinity also resulted when guanidine was located in the meta position (**4**) and when the phenyl ring was replaced by propyl (**5**). Only the *p*-guanidinylbenzyl derivative (**6**) displayed comparable affinity to **1**.

A minimum size requirement for the substituent at the C-5 position was also apparent (Table 2), since the isopropyl (**7**) derivative was less potent than **1**. The difference in affinity was smaller for the phenyl-substituted compound (**8**) and absent when a cyclohexylmethyl group (**9**) was present. Tolerance of this latter substituent allowed introduction of more polar isosteric groups, such as piperidin-1-ylmethyl (**10**), but the resulting compounds were generally less active.

While initial examples had indicated a requirement for a suitably located guanidine substituent, other basic groups were at least as effective. For instance, **11,** obtained on acylation of the N-1 aniline with glycine, was not significantly different in affinity from **1**. Marginally higher affinity was achieved for the valine derivative **12**. The cyclic guanidine-containing **13** showed a similar trend toward greater PTH_1R affinity.

The synthetic route readily allowed introduction of substituents on the benzo-fused ring, with location of a methyl group in the C-8 position (**14**−**16**) proving particularly beneficial. Around 5-fold greater affinity relative to the unsubstituted analogues (**1**, **12**, and **13**) was realized with this change, irrespective of the nature of the basic group on the N-1 substituent. The 8-methyl substituent was retained while exploring the effect of alternative basic groups, leading to the 2-imidazolylalkyl-containing derivative **17**, wherein the PTH_1R affinity was around 4 nM. Still higher affinity was achieved by increasing the lipophilic character of the N-3 substituent, but the high molecular weight and physicochemical properties of derivatives of this type such as **18** did not satisfy our preferred compound profile. Accordingly, we considered less bulky groups while retaining substituents elsewhere that had conferred high affinity in earlier compounds. For instance, the methyl analogue **19**, in which the bulk in this position was greatly reduced relative to the benzyl substituent present in **17**, was only 3-fold less potent. Altering the length of carbon chain and linking the imidazol-2-yl group of **19** with the anilide did not offer any particular advantage, as judged by lower affinity evident for

Table 2. Biological Data for N-1 Anilino-Substituted 1,3,4-Benzotriazepines[a]



compd	R_1	R_2	R_3	R_4	X	PTH_1[b]
7	Bn	*i*-Pr	H	H	C(=NH)NH_2	43% (10μM)[c]
8	Bn	Ph	H	H	C(=NH)NH_2	5.47
9	Bn	*c*-$C_6H_{11}CH_2$	H	H	C(=NH)NH_2	6.25
10	Bn	piperidin-1-yl-CH_2	H	H	C(=NH)NH_2	27% (10μM)[c]
11	Bn	*c*-C_6H_{11}	H	H	$COCH_2NH_2$	6.17 ± 0.10
12	Bn	*c*-C_6H_{11}	H	H	COCH(*i*-Pr)NH_2	6.42 ± 0.12
13	Bn	*c*-C_6H_{11}	H	H	4,5-dihydro-1*H*-imidazol-2-yl	6.54 ± 0.06
14	Bn	*c*-C_6H_{11}	H	Me	C(=NH)NH_2	6.81 ± 0.03
15	Bn	*c*-C_6H_{11}	H	Me	COCH(*i*-Pr)NH_2	6.95 ± 0.05
16	Bn	*c*-C_6H_{11}	H	Me	4,5-dihydro-1*H*-imidazol-2-yl	7.15 ± 0.02
17	Bn	*c*-C_6H_{11}	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	8.42 ± 0.10
18	3-benzosuberanyl	*c*-C_6H_{11}	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	9.04 ± 0.06
19	Me	*c*-C_6H_{11}	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	7.88 ± 0.16
20	Me	*c*-C_6H_{11}	H	Me	CH_2-1*H*-imidazol-2-yl	7.30 ± 0.08
21	Me	*c*-C_6H_{11}	H	Me	$(CH_2)_3$-1*H*-imidazol-2-yl	7.69 ± 0.04
22	*n*-Pr	*c*-C_6H_{11}	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	8.17 ± 0.14
23	*i*-Pr	*c*-C_6H_{11}	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	8.45 ± 0.13
24	Me	*c*-C_6H_{11}	BnNH	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	8.84 ± 0.11
25	Me	*c*-C_6H_{11}	BnO	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	8.74 ± 0.05
26	3-benzosuberanyl	4-tetrahydropyranyl	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	8.17 ± 0.06
27	Me	4-tetrahydropyranyl	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	6.21
28	3,4-dihydro-2*H*-1,5-benzodioxepin-3-yl	*c*-C_6H_{11}	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	8.06 ± 0.31
29	$MeO(CH_2)_2$	*c*-C_6H_{11}	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	7.08 ± 0.02
30	4-tetrahydropyranyl	*c*-C_6H_{11}	H	Me	$(CH_2)_2$-1*H*-imidazol-2-yl	8.18 ± 0.11

[a] Data were generally obtained from at least three separate experiments. Where no SEM is recorded, the data were obtained from two experiments. [b] pK_I ± SEM values obtained from competition with [^{125}I]-[Nle,^{8,18} Tyr^{34}]-hPTH(1−34) for recombinant $hPTH_1Rs$ in HEK293 cell membranes. [c] Where pK_I could not be determined, the percentage inhibition achieved at the given concentration is recorded.

20 and **21**. The reduced affinity encountered on replacing benzyl (**17**) by methyl (**19**) was largely restored when bulkier aliphatic groups were used (**22**, **23**), with the isopropyl-containing **23** being as potent as **17**. An alternative approach involving further substitution on the benzo-fused ring (**24**, **25**) provided compounds that were at least as potent, even though only an N-3 methyl group was present. Moreover, the beneficial effect on affinity from changes of this type appeared to be over and above that resulting from location of a methyl group at the C-8 position.

Compounds were now available, up to 1000-fold more potent at $hPTH_1Rs$ than our initial lead (**1**), that had mostly stemmed from the introduction of relatively more lipophilic substituents. However, this increase in lipophilicicity was tempered by exploring the effects of analogous heteroatom-containing groups. Of the compounds containing a C-5 tetrahydropyran-4-yl group (**26**, **27**), the N-3 methyl derivative **27** suffered a relatively larger reduction in affinity with respect to its carbon counterpart (**19**) in comparison with **26** and **18**. For substituents in the N-3 position, comparison with a more lipophilic analogue **18** was only possible in the case of **28**, whereby the latter compound was evidently 10-fold less potent. Of the remaining compounds of this type (**29**, **30**) the tetrahydropyran-4-yl-containing compound **30** was the more effective, displaying <10 nM affinity at $hPTH_1Rs$.

On the basis of the PTH_1R affinity displayed by many examples of this class of compounds, we had made considerable progress toward accomplishing our initial aims. Where examined, the compounds also behaved as antagonists as judged by their activity profile in cell-based functional assays. For instance, **19** had no effect on cAMP production on its own at up to 10 μM in SaOS-2 cells (a human osteocarcoma cell line expressing endogenous PTH_1Rs) but produced a dose-dependent rightward shift of the PTHrP(1−34) concentration−effect curve (pK_B = 7.55 ± 0.40). Similarly, with MC3T3-E1 cells (a mouse osteoblastic cell line expressing endogenous PTH_1Rs), **19** did not stimulate cAMP output at 3 μM but antagonized PTHrP-evoked cAMP production, albeit with around 20-fold lower potency than in the SaOS-2 cell-based assay (Table 3). Moreover, **19** was shown to be at least 100-fold selective toward PTH_1Rs over PTH_2Rs, based on comparison of its potency in inhibiting TIP(1−39) stimulated cAMP production in CHO-K1 cell membranes bearing recombinant $hPTH_2Rs$ and PTH-(1−34)-stimulated cAMP in HEK293 cell membranes expressing recombinant $hPTH_1Rs$ (pA_2 = 7.77 ± 0.20).

Table 3. Biological Activity at PTH Receptors from Cell-Based Assays[a]

compd	PTH_1[b]	PTH_1[c]	PTH_2[d]
PTH(1−34)	7.95 ± 0.07[e]	7.98 ± 0.16[e]	8.59 ± 0.38[e]
PTHrP(1−34)	8.60 ± 0.19[e]	8.70 ± 0.22[e]	<5[e]
TIP(1−39)	<5[e]	<5[e]	9.62 ± 0.09[e]
[Nle^{30}]-TIP(7−39)	8.35 ± 0.19[f]	8.18 ± 0.35[f]	7.55 ± 0.28[g]
19	7.71 ± 0.33[f]	6.29 ± 0.12[f]	5.54 ± 0.12[g]

[a] cAMP accumulation in cells expressing PTH receptors. Data were obtained from at least three separate experiments. [b] SaOS-2 cells expressing wild-type, $hPTH_1Rs$. [c] MC3T3-E1 cells expressing wild-type, mouse PTH_1Rs. [d] CHO-K1 cells expressing recombinant, $hPTH_2Rs$. [e] pEC_{50} ± SEM values. [f] pA_2 ± SEM determined from shift of PTHrP(1−34) concentration−effect curve at 3 μM. [g] pA_2 ± SEM determined from shift of TIP(1−39) concentration−effect curve at 10 μM.

Species-dependent activity has previously been encountered for other non-peptide antagonists of type B GPCRs, including those for the calcitonin gene-related peptide (CGRP) receptor[17]

(a heterodimeric receptor comprising the calcitonin receptor-like receptor (CRLR) and receptor activating modifying protein (RAMP) 1). Although RAMPs have not so far been strongly associated with PTH_1Rs, it is becoming increasingly clear that they are often coexpressed with type B GPCRs[18,19] and can have a considerable influence on receptor pharmacology,[20] including ligand binding.[21] Thus, the activity of compound **19** in the PTH_1R cell-based assays may be due to differences in the sequences of the respective PTH_1Rs and/or associated RAMPs.

While the wider receptor selectivity and ADME characteristics are beyond the scope of this Letter, we have shown that 1,3,4-benzotriazepines are potent PTH_1R antagonists. The SAR that has been established so far has guided our subsequent effort in pursuit of compounds having the appropriate balance of interspecies PTH_1R affinity and ADME properties to establish proof of concept for a non-peptide PTH_1R antagonist as a novel treatment for bone metastases, hypercalcaemia, cachexia, and hyperparathyroidism.

Supporting Information Available: Biological testing methods, ^{1}H NMR data, experimental procedures for the preparation of compounds, and elemental analysis results of compounds **1**−**30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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