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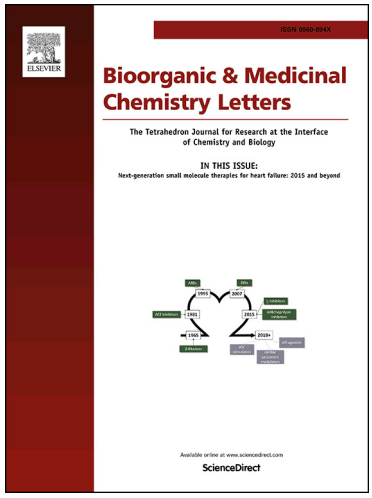

Discovery of novel PTHR1 antagonists: Design, synthesis, and structure activity relationships

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Discovery of novel PTHR1 antagonists: Design, synthesis, and structure activity relationships

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ABSTRACT

The discovery and optimization of a novel series of PTHR1 antagonists are described. Starting from known PTHR1 antagonists, we identified more potent 1,4-benzodiazepin-2-one derivatives by means of a scaffold-hopping approach. The representative compound **23** (**DS08210767**) exhibited nanomolar-level PTHR1 antagonist activity and potential oral bioavailability in a pharmacokinetic study.

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Parathyroid hormone (PTH) is an 84-amino acid polypeptide that is a principal regulator of bone remodeling and blood calcium concentration in the human body. It is mainly secreted by the parathyroid glands in response to the levels of serum calcium and phosphate through the PTH type 1 receptor (PTHR1) in the bone and kidneys.[1, 2] PTHR1 is a member of the class B family of the heptahelical G protein-coupled receptors (GPCRs). The activation of PTHR1 leads to a cascade of multiple downstream signaling pathways induced by the stimulation of adenylate cyclase-cyclic AMP (cAMP) and phospholipase C (PLC), similar to other GPCRs. As in the case of hyperparathyroidism, excess circulating PTH causes hypercalcemic conditions, which may be severely debilitating or potentially lethal.[3] Therefore, PTHR1 antagonists may have potential clinical applications in disorders of calcium metabolism.

However, the development of small-molecule ligands for PTHR1 has been challenging because the molecule is a member of the class B family of GPCRs.[4] Recently, the small-molecule PTHR1 agonist PCO371,[5] the first clinical example of an orally active class B GPCR ligand, was reported. In addition, several small-molecule PTHR1 antagonists, compound **1**[6] (SW106) and compounds **2**–**5**,[7-10] have been reported so far, although no information is available on their clinical development or pharmacokinetic (PK) profile (Fig. 1). We therefore sought to identify a suitable lead compound for a program aimed at developing an orally available antagonist of PTHR1. To this end, we utilized a scaffold-hopping approach based on known PTHR1 antagonists. Our results found that the use of privileged scaffolds based on seven-membered rings led to a novel highly potent PTHR1 antagonist with a good PK profile.

To start our research, we focused on 1,3,4-benzotriazepine-based compounds **3**–**5** described in the patent application from the James Black Foundation (JBF) as our starting compounds. Compound **5** was found to have antagonist activity at micromolar concentrations against PTHR1 (IC_{50} = 9.4 μM), as evaluated using our in-house functional assay,[11] and showed strong lipophilicity (cLogP: 6.9, Log*D*: 4.2).[12]

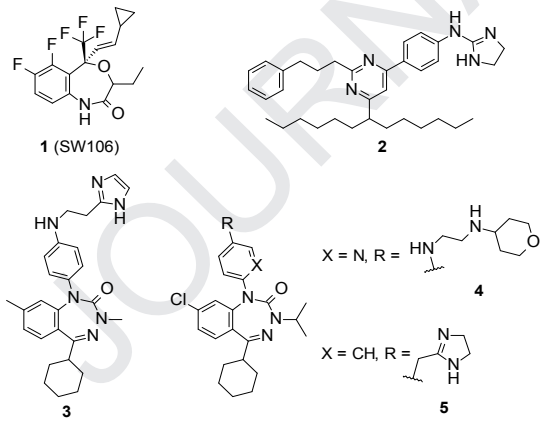
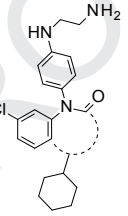


Figure. 1. Chemical structure of disclosed PTHR1 antagonists.

With the aim of identifying novel orally available PTHR1 antagonists, we explored a new core ring scaffold based on seven-membered privileged structures, because some privileged structures have offered a promising means through which new lead compounds with good drug-like properties may be identified.[13] For the structure of the linker and the basic moiety, an *N*-phenylethylenediamine unit hybridized compound, **4** and **5**, were chosen to reduce the lipophilicity and simplify the synthesis. In addition, we expected that the flexible linker would allow the molecule to adopt various relative arrangements between the upper basic parts and lower the core ring structure, thereby increasing the possibility that the molecule would bind appropriately to the PTHR1 receptor.

We initiated SAR studies of the core ring scaffold by replacing it with privileged fused [7-6] ring systems.[14] As shown in Table 1, conversion to compound **6** with 1,4-benzodiazepin-2-one resulted in antagonist activity with an IC_{50} value[11] of 38 μM, which gave satisfactory results in terms of activity and lipophilicity at this stage. In contrast, compounds **7** and **8** exhibited a substantial loss of activity. These data convinced us that we should keep the 1,4-benzodiazepin-2-one core for further structural modification. We therefore examined various 3-substitutions of compound **6**.

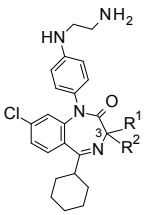
Table 1. Core-fused [7-6] bicycle SAR.



Compound	Core ring	IC_{50} (μM)[11]	cLogP/Log*D*[12]
6		38	3.6/1.7
7		>100	4.0/1.4
8		>100	2.9/NT

The various substituents at the 3-position of the 1,4-benzodiazepin-2-one ring of compound **6** are summarized in Table 2. With regard to substituent R^1, the introduction of a dimethyl amino group (**9**) was approximately equipotent with compound **6**. In contrast, the activity of the *i*-Pr derivative (**10**) was >2-fold higher than that of the non-substituted parent **6**. In addition, further improvement of the antagonistic activity was realized by compound **11** quaternized by the addition of a methyl group as a substituent at R^2. Finally, we synthesized compound **12** to investigate a *gem*-dimethyl group, and the most appropriate substituent at the 3-position proved to be this *gem*-dimethyl group (**12**).

Table 2. SAR of 3-substituted derivatives of 1,4-benzodiazepin-2-one.

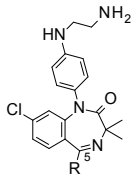


Compound	R^1	R^2	IC_{50} (μM)[11]
6	H	H	38
9[a]	NMe_2	H	44
10[a]	*i*-Pr	H	14
11[a]	*i*-Pr	Me	6.8
12	Me	Me	3.5

[a] **9**–**11**; a racemate

Next, we investigated the derivatization of various substituents at the 5-position (Table 3). The conversion to a simple *i*-Bu analog (**13**) was significantly less potent than compound **12**. Furthermore, a sterically hindered substituent, such as the 1-methylcyclohexyl group (**14**), was not tolerated, and in the case of a planar substituent, such as a phenyl group (**15**), was considerably less effective. Consequently, the cyclohexyl group (**12**) is suitable for the 5-substituent of the 1,4-benzodiazepin-2-one derivatives; it is likely that moderate space occupancy is important for PTHR1 recognition.

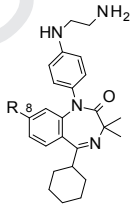
Table 3. SAR of 5-substituents of 1,4-benzodiazepin-2-one.



Compound	R	IC_{50} (μM)[11]
12	cyclohexyl	3.5
13	*i*-Bu	23
14	1-methylcyclohexyl	16
15	Ph	45

To gain insights for further improvement of the antagonistic activity, we explored the effect of various substituents on the phenyl ring of 1,4-benzodiazepin-2-one. The search for the optimal position of the chlorine atom on the phenyl ring revealed that a significant decrease in activity was observed at all sites except the 8-position (data not shown). Therefore, further derivatization of the substituent at the 8-position was performed, as shown in Table 4. The 8-methyl derivative (**16**) showed slightly weaker activity, whereas the activity of the 8-methoxy derivative (**17**) was not acceptable. In contrast, consistent with the nitrile group being a halogen bioisostere,[15] interchanging the chloride with a nitrile (**12**→ **18**) resulted in a 5-fold boost in activity. Furthermore, the lipophilicity of compound **18** (cLogP: 3.4, Log*D*: 2.1) was considerably lower than that of **12** (cLogP: 4.7, Log*D*: 3.0).

Table 4. Effect of various substituents on the phenyl ring of 1,4-benzodiazepin-2-one.

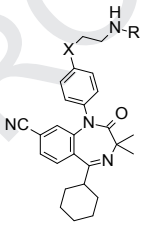


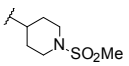
Compound	R	IC_{50} (μM)[11]
12	Cl	3.5
16	Me	6.1
17	OMe	14
18	CN	0.73

Subsequently, we performed and investigation of the linker moiety (Table 5). Compound **19**, with a diamine linker one carbon unit longer than compound **18**, exhibited 5-fold weaker activity than **18**. In addition, the conversion of the aniline nitrogen atom (**18**) to an oxygen (**20**) or carbon atom (**21**) did not result in any enhancement of activity. These results indicated that the aniline nitrogen atom on the linker moiety was important for the activity and that a two-carbon diamine linker was optimal. Furthermore, we introduced substituents on to the terminal amine (compounds **22**–**24**) for further optimization of the activity. Compound **22**, with a cyclohexane ring, led to a slight decrease in potency, although the potency was still tolerable. In contrast, the introduction of tetrahydropyran and 1-methanesulfonyl-piperidine boosted the activity by more than 4- to 8-fold, and the obtained compounds **23** (**DS08210767**) and **24**, exhibited IC_{50} values of 90 and 180 nM.

We therefore succeeded in identifying a highly potent novel PTHR1 antagonist, **DS08210767**, with a 1,4-benzodiazepin-2-one structure on the core ring, which was enhanced by more than 100-fold in comparison with compound **5**.

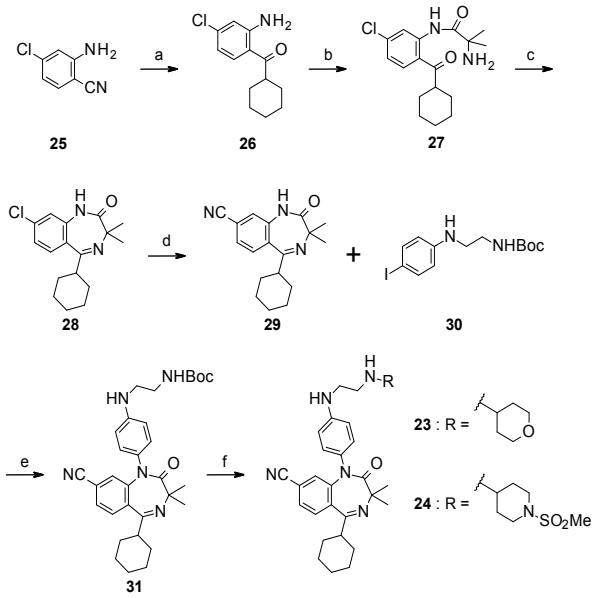
Table 5. Optimization of the linker moiety and substituents on the terminal amine.



Compound	X	R	IC_{50} (μM)[11]
18	NH	H	0.73
19	NH-CH_2-	H	4.1
20	O	H	4.0
21	C	H	7.6
22	NH		2.3
23 (DS08210767)	NH		0.09
24	NH		0.18

Following the discovery of **DS08210767**, with excellent in vitro PTHR1 antagonist activity, appropriate lipophilicity, and high solubility, we next investigated the PK profile in Sprague-Dawley rats (Table 6). In this study, **DS08210767** exhibited high plasma exposure in rats and was considered to have a suitable profile as an orally administered agent.

The preparation of the representative compounds **DS08210767** and **24** is shown in Scheme 1. The 8-cyano-1,4-benzodiazepin-2-one core **29** was prepared by a four-step procedure from starting material **25**. The Grignard reaction with nitrile **25** afforded a ketone **26**, which was condensed with 2-amino-2-methyl-propanoyl chloride hydrochloride[16] to provide the cyclization precursor **27**. Then, intramolecular cyclization, induced by refluxing compound **27** in *n*-butanol in the presence of triethylamine as a base yielded the 1,4-benzodiazepin-2-one ring **28**, which was then converted to the intermediate **29** via the palladium-catalyzed cyanation[17] of aryl chloride **28**. Thus, the copper-catalyzed amidation[18] of aryl iodide **30**[19], prepared from 4-iodo-aniline with **29**, was performed to provide the corresponding diamine analog **31**. After removal of the Boc group from compound **31** under acidic conditions, the resultant amine was converted to the target compounds **DS08210767** and **24** by reductive amination with the corresponding ketone.



Scheme 1. Synthesis of representative compounds **23** (**DS08210767**) and **24**. Reagents and conditions: (a) cyclohexylmagnesium bromide, THF, 0 °C to rt, 87%; (b) 2-amino-2-methyl-propanoyl chloride; hydrochloride, MeCN, rt; (c) triethylamine, 1-butanol, reflux, 65% (for two steps); (d) *t*BuXphos Pd G1, *t*BuXPhos, KOAc, potassium hexacyanoferrate(II) trihydrate, dioxane-H_2O, 100 °C, 76%; (e) CuI, *trans*-*N*,*N*'-dimethylcyclohexane-1,2-diamine, K_2CO_3, dioxane, 100 °C, 93%; (f) (i) TFA, CH_2Cl_2, rt, 93%; (ii) ketone (for **23**, tetrahydro-4H-pyran-4-one; for **24**, 1-*N*-(methylsulfonyl)-4-piperidinone, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 97% (for **23**), 85% (for **24**).

In summary, we successfully identified the novel 1,4-benzodiazepin-2-one-based PTHR1 antagonist **DS08210767** by means of a scaffold-hopping approach based on the structure reported in JBF's patent. The compound exhibited potent antagonist activity and an ADME/PK profile that suggested good oral bioavailability.
DS08210767 is expected to be useful for studying disorders of calcium metabolism such as hypercalcemia and bone disease.

Table 6. Physicochemical properties and Pharmacokinetic parameters of **DS08210767**.

Log D[a]	Solubility JP1/JP2[b] (μg/mL)	MS[c] (%)	PB[d] free (%)	CL[e] (mL/min/kg)	Vd[e] (L/kg)	$T_{1/2}$[f] (h)	AUClast[f] (h*μg/mL)	%F
2.9	760/500	28	2.9	46.8	4.7	4.23	1.94	27

[a] The distribution coefficients (log *D*) were measured between 1-octanol and phosphate buffered saline (pH 7.4).
[b] JP1/JP2: Japanese pharmacopoeia first/second test fluid (pH = 1.2/6.8).
[c] MS: metabolic stability, remaining (%) of compound **23** after 0.5 h of incubation with rat liver microsomes.
[d] PB: protein binding, unbound fractions (%) in rat plasma.
[e] 1 mg/kg intravenous administration in male Sprague-Dawley rats (n = 2). Dosing formulation: DMA/Tween80/PBS (1:1:8).
[f] 10 mg/kg oral administration in male Sprague-Dawley rats (n = 3). Dosing formulation: PG/Tween (4:1).

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11. IC_{50} values were measured by cAMP assay on human PTHR1 receptor-expressing CHO-K1 cells and determined in a 8-point dose-response curve in quadruplicate measurements at each concentration. For the detailed experimental procedures, please see Supplementary data.
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19. For the preparation of compound **30**, please see Supplementary data.

Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.

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