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Difluoroethylamines as an amide isostere in inhibitors of cathepsin K

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ABSTRACT

The trifluoroethylamine group found in cathepsin K inhibitors like odanacatib can be replaced by a difluoroethylamine group. This change increased the basicity of the nitrogen which positively impacted the log *D*. This translated into an improved oral bioavailability in pre-clinical species. Difluoroethylamine compounds exhibit a similar potency against cathepsin K and selectivity profile against other cathepsins when compared to trifluoroethylamine analogs.

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bones. A direct consequence of this disease is an increased risk of fractures. Inhibition of cathepsin K (Cat K) may offer a potential cure for osteoporosis.[1] Cat K, a cysteine protease, plays an important role in bone degradation.[2] Several Cat K inhibitors from various structural classes had been reported in the literature.[3] The amide bond is a frequent chemical motif amongst these inhibitors and metabolic liabilities associated to an amide bond are well-known. A successful replacement of an amide bond by a trifluoroethylamine was recently reported, leading to the discovery of odanacatib, now in phase III clinical trial for post-menopausal osteoporosis (Fig. 1).[4,5] The trifluoromethyl moiety represents a fine balance between electron-withdrawing character, metabolic stability and adequate steric bulk. However odanacatib is a highly crystalline molecule with low aqueous solubility. As a result, the oral bioavailability of the crystalline form as a suspension in methocel was low across pre-clinical species (⩽10%). We now report a modification to the CF_3 group (Fig. 1) to modulate the physical properties like pK_a in an attempt to further improve on the oral bioavailability.

Our working hypothesis was that by reducing the number of fluorines to two (CF_2H vs CF_3), the chemical and metabolic stabilities of the proposed inhibitors should not be affected. This would however result in a more basic series of inhibitors. To fully assess the impact of the modification, measurements of physical properties like aqueous solubility, pK_a, log D_7 and pharmacokinetic

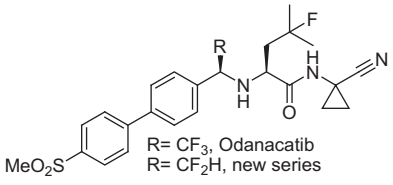

Figure 1. Odanacatib and a new series of Cat K inhibitors.

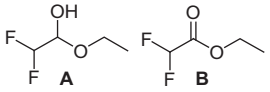

Figure 2. Starting materials used for the synthesis of difluoroethylamine analogs.

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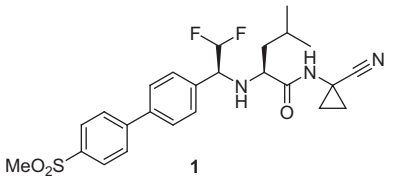
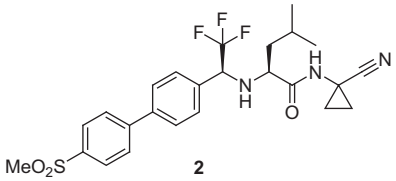
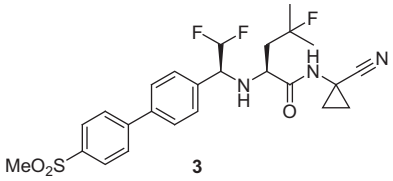
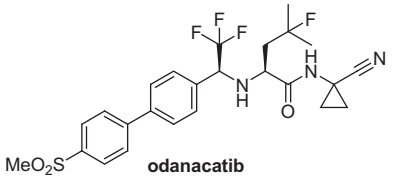
Table 1
Comparison of physical properties and pharmacokinetics

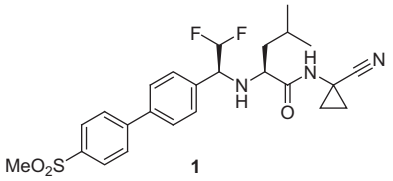
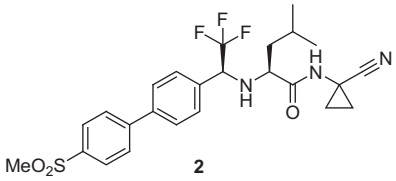
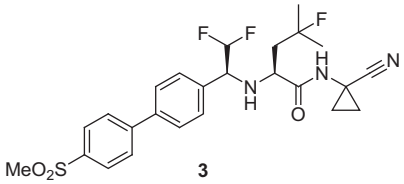
Compound	Aq solubility (mg/mL)	pK_a	log D_7	PK in rats (%F, $t_{1/2}$)[a]	PK in dogs (%F, $t_{1/2}$)[b]
 **1**	<0.01	2.7	0.11	100%; 0.8 h	na
 **2**	<0.01	<2.0	3.53	4%; 2.6 h	na
 **3**	na	na	na	22%; 3.8 h	23%; 36 h
 **odanacatib**	na	na	na	8%; 6 h[c]	6%; 57 h[c]

[a] PO dose = 10 mg/kg (1% methocel), IV dose = 2 mg/kg (60% PEG-200).
[b] PO dose = 5 mg/kg (1% methocel), IV dose = 1 mg/kg (60% PEG-200).
[c] For PO, vehicle is 0.5% methocel + 0.2% SDS.

studies would be performed. The successful formation of chemically stable salts would also differentiate the difluoroethylamine compounds from the trifluoroethylamine ones.

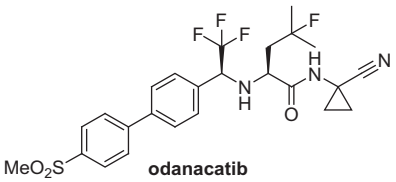
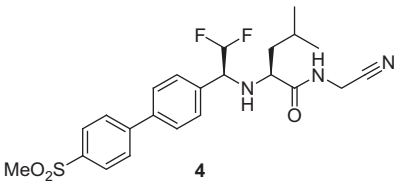
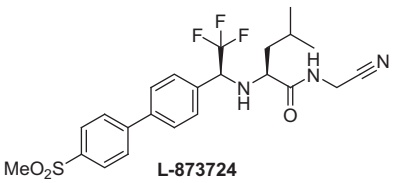
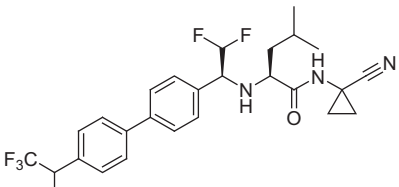
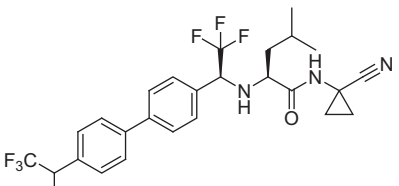
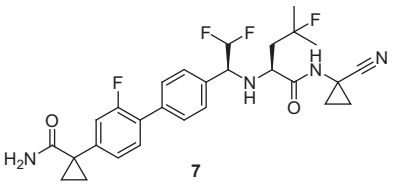
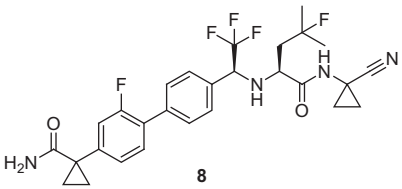
The difluoroethylamine compounds were synthesized following the chemical routes developed for odanacatib.[6–8] The only difference resided in the initial starting material. Syntheses were

Table 2
Comparative in vitro profile of CF_2H versus CF_3 analogs

Structure	Hrab CatK[a] IC_{50}[b] (nM)[15]	Cat B/K	Cat F/K	Cat L/K	Cat S/K	'Corrected' Bone Res IC_{50} (nM)[16]
 **1**	0.8	>11,915	645	894	1142	10
 **2**	0.3	23,561	370	1389	809	3
 **3**	1.0	2297	1924	6326	210	9

(*continued on next page*)

Table 2 (*continued*)

Structure	Hrab CatK[a] IC_{50}[b] (nM)[15]	Cat B/K	Cat F/K	Cat L/K	Cat S/K	'Corrected' Bone Res IC_{50} (nM)[16]
 **odanacatib**	0.2	5170	3975	14,975	300	5
 **4**	0.3	>32,589	822	869	2001	15*
 **L-873724**	0.2	26,195	480	1320	890	3
 **5**	1.9	>5184	126	785	813	32
 **6**	1.6	5978	na	506	311	21
 **7**	0.3	7234	2339	12,670	2878	4*
 **8**	0.3	4400	1383	9368	1204	2*

na = not available.

[a] Humanized rabbit enzyme.

[b] IC_{50}'s are an average of at least two independent titrations, except values marked with * which are $n = 1$.

initiated with either difluoroacetaldehyde ethyl hemiacetal **A** or difluoroacetic acid ethyl ester **B** (Fig. 2).

Physical properties of close analogs of odanacatib (aqueous solubility, pK_a and log D at pH 7) as well as pharmacokinetics are summarized in Table 1. Two pairs of compounds (CF_2H vs CF_3) were available for comparison. Although **1** and **2** both exhibited low aqueous solubility,[9] there was an impact on measured pK_a's. There is almost one log unit difference between **1** and **2**.[10] The effect on log D was noticeable.[11] When measured at pH 7, there was more than three log units difference between **1** and **2**. By comparing oral bioavailability (%F) of compounds **1** and **2**, it was clear that compound **1** was better absorbed (F **1** = 100% vs F **2** = 4%). The same holds for compound **3** and odanacatib. Compound **3** exhibited an increased bioavailability in rats and dogs compared to odanacatib. Besides physical properties, it was also important to evaluate this series of compounds for potency and selectivity. As

shown in Table 2, five pairs of compounds comprising various P1, P2 and P3 groups[12] exhibited comparable in vitro potency against Cat K, selectivity profile against other cathepsins and potency in the functional bone resorption assay in rabbit osteoclasts.[13] CF_2H compounds (**1**, **3**, **4**, **5** and **7**) displayed similar potency and selectivity when compared to the CF_3 derivatives (L-873724, **2**, odanacatib, **6** and **8**).

Increasing the basicity of the nitrogen allows the formation of chemically stable salts of **1** with several acids such as 2-naphthalenesulfonic acid, hydrochloric acid, sulfuric acid, methanesulfonic acid and *p*-toluenesulfonic acid.[14] Rat pharmacokinetic studies were conducted on these salts but provided no advantage over the neutral form.

In summary, we have increased the basicity of the nitrogen of trifluoroethylamine compounds by preparing difluoroethylamine analogs (CF_2H). This change resulted in an increased log D_7 and positively impacted the oral bioavailability. It is noteworthy that this modification provided inhibitors with similar potency against Cat K and selectivity against other cathepsins.

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9. *Solubility measurements*: Equilibrium solubility measurements were carried out by stirring the compounds, prepared at a concentration range of 5–10 mg/mL in water for 16 h at room temperature in the absence of light. The supernatant was recovered by centrifugation (15,000 rpm) and the excess solids were recovered for X-ray powder diffraction analysis. Solubility measurements were determined by HPLC. *X-ray powder diffraction*: XRPD patterns were measured using a Bruker Discover D8, Vantec-1 PSD detector, Cu Kα source at a generator power of 35 kV and 45 mA. A detector-scan continuous mode scan (transmission mode) was used with a range of 5–40° 2θ with a step size of 0.014° and 0.2 s time per step. The step time was 351 s (23 min total run time). The samples were measured on a kapton plate.
10. The pK_a values were determined by potentiometric titrations with spectrophotometric analysis. The titrations were performed with the Sirius GLpKa/D-PAS using a double junction electrode. Each sample was dissolved in DMSO to create a stock solution of 20.0 mg/mL. An aliquot of each stock solution (0.01 mL) was added to a titration vial and diluted up to 10 mL of 50% MeOH:50% ionic strength adjusted (ISA) H_2O (0.15 M KCl in H_2O). The starting pH of each solution was adjusted with 0.5 M HCl. Each solution was titrated from about pH 2–11 at 25 °C in ~50% MeOH, followed by subsequent dilutions to ~40, and ~30% MeOH solutions and retitrations, all in the same vial. The pK_a value in ISA H_2O was determined by Yasuda–Shedlovsky extrapolation to 0% MeOH. The pK_a values were calculated using RefinementPro v.2.2 software.
11. The chromatographic system consists of an Agilent 1200 HPLC system. The separations are carried out on a XTerra MS C_{18}, 30 mm × 3.0 mm I.D., 3.5 μm, 125 Å (Waters Corporation, USA). The mobile phase consists of phosphate buffered saline at pH 7 (mobile phase A) and acetonitrile (mobile phase B). The column oven temperature is set to 30 °C. The HPLC analysis begins with an isocratic step of 0.2 min at 5% B at 1.5 mL/min, followed by a gradient from 5% to 98% B in 1.0 min at 1.5 mL/min. A second isocratic step of 0.2 min at 98% B with a changing flow rate from 1.5 to 2.0 mL/min is then followed by a gradient from 98 to 5% B in 0.1 min with the flow rate changing from 2.0 to 1.5 mL/min. The equilibration time between injections is 0.2 min at 5% B. The injection volume is 5 μL and the spectrophotometric detection is set to 215, 238 and 254 nm. A 10 mM DMSO stock solution of API is delivered for analysis. A 100 μM standard solution is generated by diluting 10 μL of the 10 mM stock solution with 990 μL of diluent (10% DMSO/10% MeCN/80% MeOH, v/v/v). The chromatographic system is calibrated with a set of standards with published shake-flask log *D* values. Linear regression is used to determine the calibration line relating the retention time to log *D* for the calibration standards. This line is then used to determine the HT HPLC log $D_{7.0}$ value of API from the measured retention time by the HPLC/DAD analysis of the 100 μM standard solution.
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13. Assay conditions described in: Falgueyret, J.-P.; Oballa, R. M.; Okamoto, O.; Wesolowski, G.; Aubin, Y.; Rydzewki, R. M.; Prasit, P.; Riendeau, D.; Rodan, S. V.; Percival, M. D. *J. Med. Chem.* **2001**, *44*, 94–104.
14. *General procedure for the salt formation*: To an CH_3CN solution of a CF_2H compound (~0.1 M) was added a 1.0 M CH_3CN solution of the acid (1.0 equiv). The solvent was evaporated. The salts could be used as such, or were dissolved in CH_2Cl_2 and precipitated with Et_2O.
15. Human Cat B, F, K, L and S enzymes. Screening conditions: Desmarais, S.; Massé, F.; Percival, M. D. *Biol. Chem.* **2009**, *390*, 941. and references cited therein.
16. Bone res IC_{50} × Hrab Cat K IC_{50}/rabbit Cat K IC_{50}. This corrects for the difference between rabbit and human potencies.