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The discovery of MK-0674, an orally bioavailable cathepsin K inhibitor

Elise Isabel [a,*], Kevin P. Bateman [a], Nathalie Chauret [a], Wanda Cromlish [a], Sylvie Desmarais [a], Le T. Duong [b], Jean-Pierre Falgueyret [a], Jacques Yves Gauthier [a], Sonia Lamontagne [a], Cheuk K. Lau [a], Serge Léger [a], Tammy LeRiche [a], Jean-François Lévesque [a], Chun Sing Li [a], Frédéric Massé [a], Daniel J. McKay [a], Christophe Mellon [a], Deborah A. Nicoll-Griffith [a], Renata M. Oballa [a], M. David Percival [a], Denis Riendeau [a], Joël Robichaud [a], Gideon A. Rodan [b], Sevgi B. Rodan [b], Carmai Seto [a], Michel Thérien [a], Vouy Linh Truong [a], Gregg Wesolowski [b], Robert N. Young [a], Robert Zamboni [a], W. Cameron Black [a]

[a] Merck Frosst Centre for Therapeutic Research, 16711 TransCanada Hwy, Kirkland, Québec, Canada H9H 3L1
[b] Department of Bone Biology and Osteoporosis, Merck Research Laboratories, West Point, PA 19846, USA

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ABSTRACT

MK-0674 is a potent and selective cathepsin K inhibitor from the same structural class as odanacatib with a comparable inhibitory potency profile against Cat K. It is orally bioavailable and exhibits long half-life in pre-clinical species. In vivo studies using deuterated MK-0674 show stereoselective epimerization of the alcohol stereocenter via an oxidation/reduction cycle. From in vitro incubations, two metabolites could be identified: the hydroxyleucine and the glucuronide conjugate which were confirmed using authentic synthetic standards.

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone tissues. This leads to bone fragility and an increased susceptibility to fractures, especially of the hip, spine and wrist. Significant risk has been reported in aging populations, particularly post-menopausal women being most susceptible. Indeed, 80% of people affected by this disease are women. Age is the highest risk factor in both men and women: up to 55% of the people over 50 are suspected to have low bone mass density or osteopenia. Because bone loss is asymptomatic, osteoporosis is often known as 'the silent thief'. As the population continues to age, the burden of osteoporosis on medical care systems will increase. According to the National Osteoporosis Foundation, in 2005 osteoporosis-related fractures were responsible for an estimated $17 billion costs in the USA. By 2025, these costs could rise as high as $25 billion.[1] Bisphosphonates, estrogen replacement therapy and parathyroid hormone are the current standard care for osteoporosis.[2,3] Because these treatments do not suit all patients and reduce approximately 50% of fracture risk, there is an urgent need for new therapies.

Approximately 15 years ago, cathepsin K (Cat K) was isolated and characterized.[4] There is strong evidence that Cat K plays a major role in bone degradation.[5,6] Many Cat K inhibitors have been reported in the literature[7] since then, including balicatib,[8] relacatib[9] and odanacatib, currently in Phase III fracture prevention trial in post-menopausal women with osteoporosis.[10] Herein, we report on the successful identification of MK-0674, an orally bioavailable inhibitor from the same structural class as odanacatib with a different metabolic profile.

Odanacatib (Fig 1), in human hepatocyte incubations, is predominantly metabolized by CYP3A4.[11] The purpose of this work was to maintain the beneficial features of odanacatib in P1 (cyclopropane) and P2 (fluoroleucine) to maintain high metabolic stability while introducing another group in P3 to allow a different

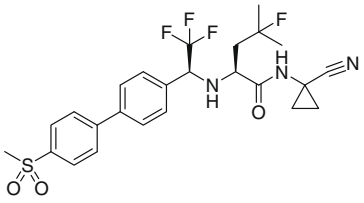

Figure 1. Odanacatib.

* Corresponding author. Address: Merck Frosst Canada, PO Box 1005, Pointe-Claire-Dorval, Québec, Canada H9R 4P8. Tel.: +1 514 428 3655; fax: +1 514 428 2624.
E-mail address: elise_isabel@merck.com (E. Isabel).

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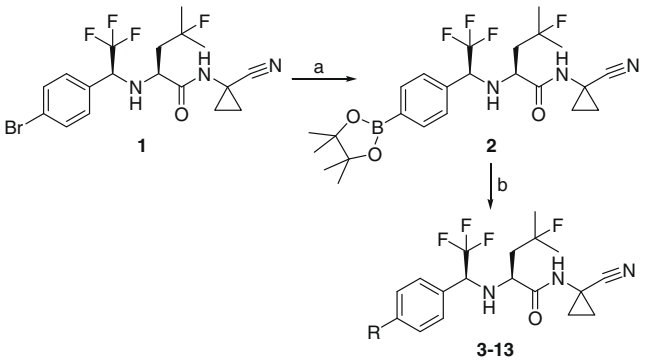
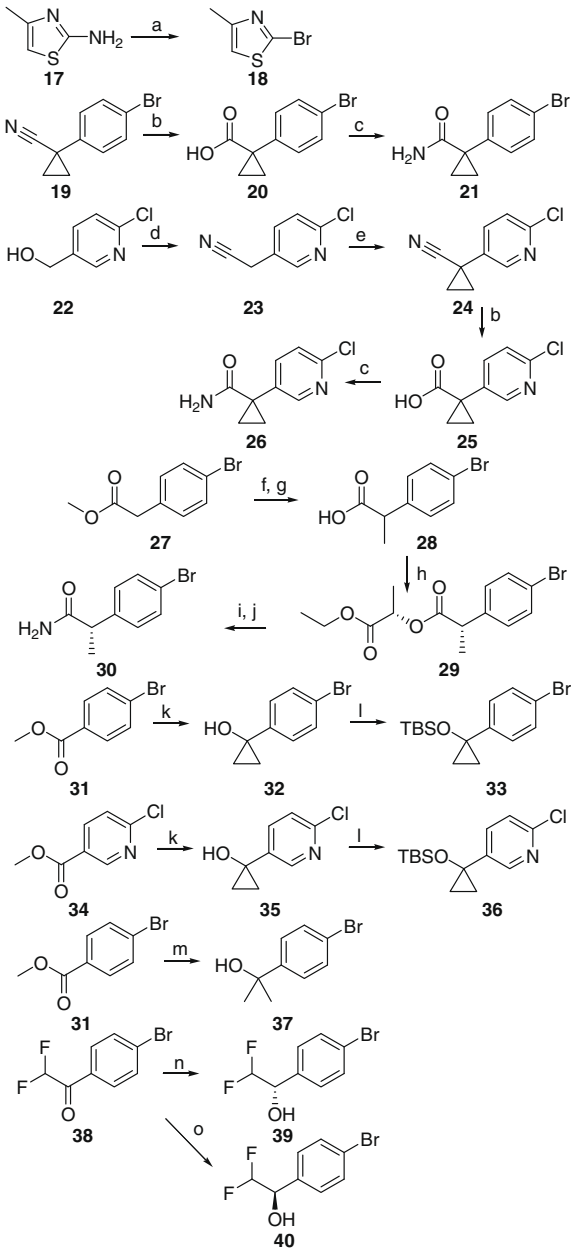
metabolism pathway without compromising its high intrinsic potency and selectivity. To do so, the biaryl moiety was substituted by a variety of functional groups: heterocycles, amides and alcohols. Several reports from our laboratories[12–15] have described efficient methods to enantiomerically synthesize large quantities of molecules in this class. Compound **1** was an intermediate from the synthesis of odanacatib and therefore, considerable amount of material was available to study SAR in the P3 region.

All analogs were synthesized using the chemistry described in Scheme 1. The required intermediates were synthesized by methods shown in Scheme 2. Compound **3** was obtained by a palladium cross-coupling between **2** and the commercially available 5-bromo-2-methanesulfonylpyridine. The same conditions were used to synthesize **4** from 6-bromoquinoline. Compound **5** was obtained from a coupling between **2** and 2-bromo-4-methylthiazole **18** which was synthesized following a well-established literature procedure[16] from the commercially available 2-amino-4-methylthiazole **17**. The syntheses of compounds **6** and **7** were initiated with 1-(4-bromo-phenyl)-cyclopropanecarbonitrile **19** and 2-chloro-5-hydroxymethylpyridine **22**, respectively. The alcohol was first mesylated and further displaced by NaCN to yield **23**. The cyclopropane **24** was obtained by a double alkylation of **23**. Then, compounds **19** and **24** were submitted to a basic hydrolysis to generate carboxylic acids **20** and **25**. The primary amides **21** and **26** were obtained after treatment with ammonia of the in situ formed mixed anhydrides. Building blocks **21** and **26** were finally reacted with **2** to provide **6** and **7**. Compound **8** was synthesized in a few steps from 4-bromophenyl acetic acid methyl ester **27**. The benzylic position was methylated with iodomethane in the presence of DMPU and LiHMDS. The carboxylic acid **28** obtained by hydrolysis of the methyl ester was then reacted with oxalyl chloride and catalytic amount of DMF. The ketene formed in situ was reacted with L-lactic acid to provide a single diastereomeric ester **29**. The ester was carefully hydrolyzed with LiOH and hydrogen peroxide to give the carboxylic acid in high enantiomeric excess. The corresponding primary amide **30** was obtained without racemization.[17] Compound **8** was obtained by reacting **30** with **2** under standard conditions. Cyclopropanols **32** and **35** were synthesized from the corresponding commercially available methyl esters **31** and **34**.[18] Palladium-catalyzed cross-coupling reactions were giving low yields when the coupling was performed on the free alcohol. Acceptable yields and cleaner material were obtained following a simple silyl ether protection with TBS. Cross-coupling between **2** and reagents **35** or **36** followed by deprotection using HF·pyridine afforded compounds **9** and **10**. Methyl Grignard addition to methyl 4-bromobenzoate **31** provided the necessary tertiary alcohol **37** to yield compound **11**.[19] Difluoroacetophenone[12] **38** was reacted neat



Scheme 1. Reagents and conditions: (a) Diboron pinacol ester (1.1 equiv), KOAc (3.1 equiv), $PdCl_2dppf$ (5 mol %), dioxane, 80 °C, 80%; (b) ArBr (1.2 equiv), 2 M Na_2CO_3 (3 equiv), $PdCl_2dppf$ (3 mol %), 80 °C or ArCl (1.2 equiv), 2 M Na_2CO_3 (2.5 equiv), $Pd(OAc)_2{\cdot}PPh_3$ (5 mol %), 1-propanol/DMF 4:1, 80 °C.

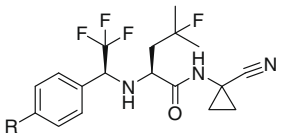


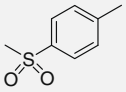
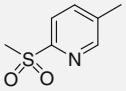
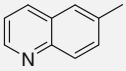
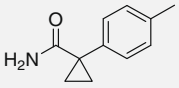
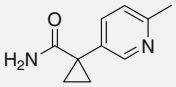
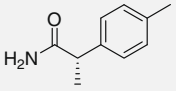
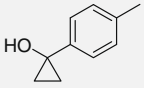
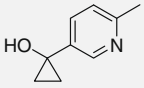
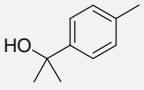
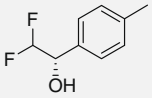
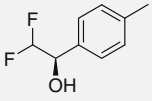
Scheme 2. Reagents and conditions: (a) 85% H_3PO_4, 65% HNO_3, $NaNO_2$ (1.3 equiv), $CuSO_4{\cdot}5H_2O$ (1.3 equiv), H_2O, 10%; (b) 25% NaOH, EtOH, 100 °C, 90%; (c) isobutyl chloroformate (1.2 equiv), Et_3N (1.3 equiv), $CHCl_3$, $NH_{3(g)}$, 0 °C; (d) (i) MsCl (1.1 equiv), Et_3N (1.2 equiv), CH_2Cl_2, −78 °C; (ii) NaCN (1.1 equiv), DMF, 80%; (e) 1-bromo-2-chloro-ethane (1.1 equiv), benzyltriethylammonium chloride (0.03 equiv), 50% NaOH (3.1 equiv), 60 °C, 88%; (f) LDA (1.1 equiv), CH_3I (4.0 equiv), −78 °C, 100%; (g) 1 M LiOH (3.5 equiv), THF/MeOH (2:1), 0 °C , 96%; (h) oxalyl chloride (1.2 equiv), DMF (0.03 equiv), toluene, *N,N*-dimethylethylamine (3.0 equiv), ethyl L-lactate (1.2 equiv), 56%; (i) 4 N LiOH (1.1 equiv), 30% H_2O_2 (2.7 equiv), MeOH, 0 °C, 67%; (j) HOBT (1.5 equiv), HATU (1.5 equiv), NH_4Cl (2.6 equiv), Hunig's base (3.9 equiv), 90%; (k) EtMgBr (3.2 equiv), $Ti(OiPr)_4$ (1.0 equiv), Et_2O, −78 °C; (l) imidazole (2.0 equiv), DMAP (0.1 equiv), TBSCl (1.1 equiv), 95%; (m) MeMgBr (3.0 equiv), THF, 0 °C, 75%; (n) *S*-alpine borane (1.2 equiv), 70%; (o) *R*-alpine borane (1.2 equiv), 90%.

with either *S*-alpine borane or *R*-alpine borane[20] to afford the enantiomerically pure reactants **39** or **40**, respectively. These were coupled under standard conditions to provide **12** and **13**.

Subtle changes to the P3 portion of odanacatib had a major impact on the potency, selectivity or pharmacokinetics of the new compounds (Table 1). By comparing compound **3** to odanacatib, it was clear that the introduction of the 3-pyridyl ring was detri-

Table 1
In vitro activity of odanacatib analogs with P3 substitution



Compound	R	Hrab Cat K[23] IC_{50} (nM)	Cat B/K	Cat F/K	Cat L/K	Cat S/K	'Corrected' Bone Res[24] IC_{50} (nM)	Comments
Odanacatib		0.2	5170	3232	14,975	300	5	
3		2.6	725	n/a	1303	28	8	10× loss in potency
4		0.4	2997	n/a	6021	44	8	Lack of selectivity against Cat S
5		0.3	4044	1985	18,753	777	14	Poor%F
6		0.2	1555	2379	11,120	766	12	Poor%F
7		0.5	3924	3442	3695	849	35	Circulating metabolite
8		0.2	1440	3592	21,332	693	35	Complicated metabolic profile
9		0.2	1420	1147	11,005	106	13	Lack of selectivity against Cat S
10		0.3	3643	2759	18,022	210	n.a.	Poor%F
11		0.2	2197	2410	28,916	715	10	Short half-life
12		0.4	1474	1600	14,119	197	7	Good candidate
13		0.4	1156	1465	11,857	243	10	Good candidate

mental: there was a 10-fold loss in potency. However, by replacing it with the quinoline of compound **4**, most of the potency was recovered but the selectivity against Cat S was greatly reduced. Heterocycles like the methylthiazole **5** provided a more interesting in vitro profile. However, the oral bioavailability was disappointing (10% in rats and 5% in the squirrel monkeys). We then turned our

Table 2
Correlation between in vitro hepatocyte incubations and pharmacokinetics of **12** and **13** (1% methocel as oral vehicle)

Species	**12**			**13**		
	In vivo		In vitro	In vivo		In vitro
	%F	$T_{1/2}$ (h)	% parent remaining	%F	$T_{1/2}$ (h)	% parent remaining
Rat	86	10	94	122	7	95
Rabbit	132	8	70	70	14	93
Rhesus monkey	139	6	88	64	11	93

attention to benzylic-substituted primary amides as an isostere of the methylsulfone. Compound **6** bearing a cyclopropane amide had a similar profile to **5**. Here again, the compound failed because of poor bioavailability in rats (8%) and squirrel monkeys (5%). It was surprising that the introduction of a pyridine to give Compound **7** did not impair the potency or the selectivity. Compound **7**, when formulated as a tosylate salt, provided 50% bioavailability in rats. However, further evaluation led to the identification of an abundant circulating metabolite in rats so it was abandoned. Similarly, compound **8** suffered from poor oral bioavailability and a complicated metabolic pathway: in pre-clinical species, circulating metabolites that were observed had not been observed in microsome or hepatocyte incubations. This poor in vitro–in vivo correlation would have led to significant developmental challenges. Since amide moieties demonstrated unsatisfactory ADME properties, we began to study benzylic alcohols in P3. All compounds incorporating an alcohol moiety at the benzylic position exhibited high potency against Cat K. Compound **9** showed poor selectivity against Cat S. Introduction of a pyridine improved this selectivity but compound **10** suffered from low bioavailability in rats (7%). The dimethyl carbinol analogue **11** was highly selective against Cat S with moderate bioavailability in rats of 24%. However **11** suffered from a half-life in rats of less than 1 h. Then, a pair of enantiomerically pure alcohols **12** and **13** was evaluated. These two compounds were potent and highly selective in in vitro assays. As well, they were potent in a functional bone resorption assay in rabbit osteoclasts.[21] Both **12** and **13** exhibited high oral bioavailabilities[22] and long half-lives in rats, rabbits and rhesus monkeys (Table 2). These results were consistent with the low levels of metabolism observed in hepatocyte incubations.

In order to better understand the metabolism of the secondary alcohol, deuterated **12D** and **13D** were prepared as a racemate and separated on a semi-preparative AD normal-phase column (Scheme 3). Rats were dosed P.O. (25 mg/kg, $n = 2$) with either **12D** or **13D**. Plasma samples were analyzed by LC–MS both on a standard C_{18} column to determine the isotopic ratio and on an OJ-R reverse-phase column to assess the diastereomeric ratio of **12** and **13**. Results of this experiment are shown in Charts 1 and 2. First of all, we observed that **12D** and **13D**[25] were losing their deuterium at a similar rate over time (Chart 1). We also noticed

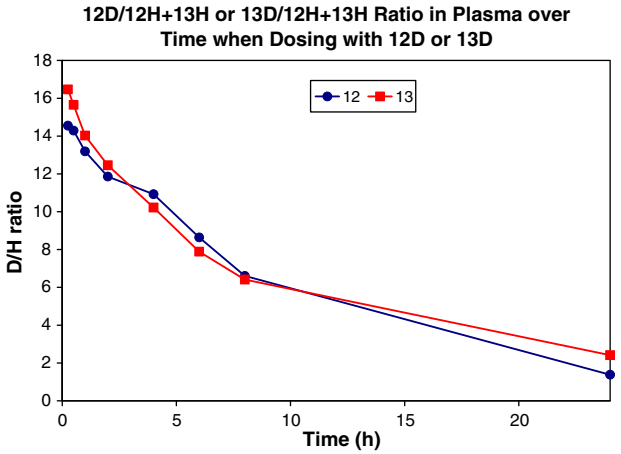


Chart 1. D/H ratio over 24 h measured in rat ($n = 2$) plasma when either **12D** or **13D** was administered P.O. Both **12D** and **13D** lose their deuterium at a similar rate.

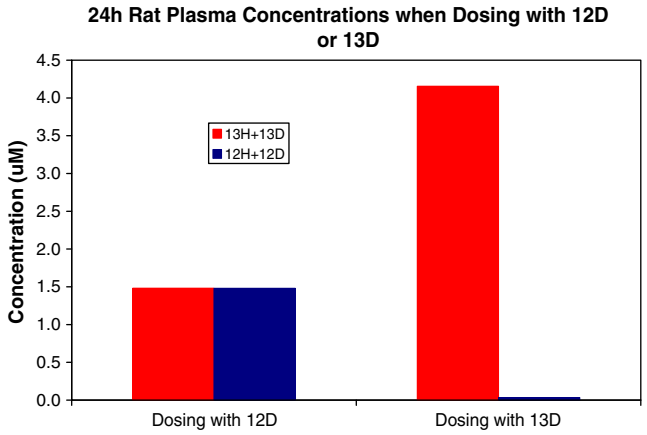
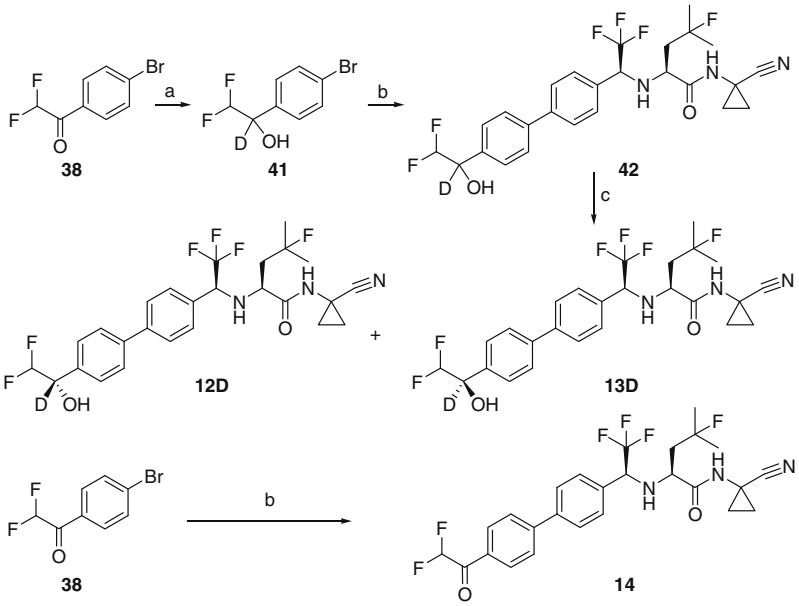


Chart 2. Concentrations of **12** and **13** in rat plasma 24 h post-dosing of either **12D** or **13D**. As **12D** loses its deuterium, it is converted to **13H**. When **13D** loses its deuterium according to the same mechanism, it is converted to **13H** as well, indicating selective reduction of an intermediate ketone.



Scheme 3. Reagents and conditions: (a) $NaBD_4$ (1.0 equiv), EtOD, 100%; (b) **2** (1.0 equiv), ArBr (1.2 equiv), 2 M Na_2CO_3 (3.0 equiv), $PdCl_2dppf$ (3 mol %), 80 °C, 87%; (c) chiralcel AD normal phase semi-preparative HPLC1:1 hexanes/EtOH isocratic, $t_R = 5.09$ and 6.53 min.

Table 3
In vitro and in vivo **13**/**12** ratio following ketone reduction

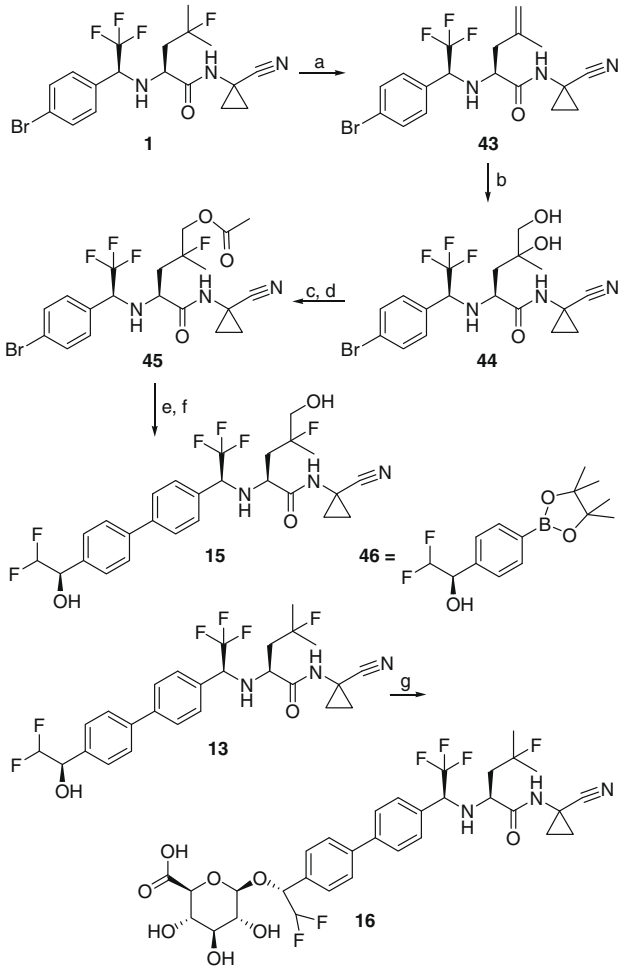
Species	In vitro **13**/**12** ratio	In vivo **13**/**12** ratio
Rat	24:1	>10:1
Dog	5:1	5:1 to 7:1
Rabbit	48:1	n.a.
Human	>200:1	n.a.

that **12D** was converted into **13H** in vivo (Chart 2). When **13D** was administered to rats, we observed that **13D** was converted to **13H**, but very little **12H** was formed. These results suggested that **12D** or **13D** were both oxidized in vivo to the corresponding ketone, followed by stereoselective reduction to **13H**. To verify this hypothesis, the ketone **14** was synthesized (Scheme 3) and dosed P.O. (25 mg/kg) and I.V. (5 mg/kg). Samples were analyzed as described above. Ketone **14** itself was not detected in any samples (earliest time point = 5 min) but both **12** and **13** were formed with a **13**:**12** ratio greater than 10:1 at all time points. Ketone **14** was incubated with hepatocytes from four species: extensive and selective reduction to **13** was observed. This reduction was most selective in human and least selective in dog (Table 3). Comparison to PK data from rat and dog showed a good correlation with in vitro incubations. The in vivo dog data was generated by dosing with pure **13D** and showed partial conversion to **12H** that was consistent with the in vitro results (P.O., t = 24 h).

Since **12** epimerized to **13** in vivo, **12** was abandoned and further profiling was done on **13**. Two metabolites were identified from incubations: hydroxyleucine **15** and the glucuronide conjugate **16**. Authentic standards were synthesized in order to confirm the structure (Scheme 4). Compound **15** was obtained after a multi-step synthesis. Compound **1** was treated with $BF_3{\cdot}Et_2O$ to afford the alkene **43**. It was dihydroxylated with catalytic osmium tetroxide to give the diol **44**. The primary alcohol was acylated, whereas the tertiary alcohol was treated with DAST to yield **45**. The aryl bromide **40** was turned into the boronic ester **46** following the procedure described above. A Suzuki cross-coupling was conducted between **45** and **46** followed by a basic hydrolysis to afford the metabolite **15**.

Compound **16** was obtained from dog liver microsome incubation[26] and isolated from the incubate according to a published method.[27] Incubations with human liver microsomes revealed that the oxidative metabolism was mainly due to CYP3A4. However, incubations with 12 different rUGTs (recombinant uridine 5'-phosphate-glucuronosyl transferase) indicated that multiple UGTs were involved in the glucuronidation process.

In conclusion, by replacing the methyl sulfone of odanacatib with a difluoromethylcarbinol, MK-0674 was identified. It fulfilled our requirements in terms of potency, selectivity, pharmacokinetics and metabolism to provide a valuable back-up. It differentiates from odanacatib since it is partially excreted via a glucuronide conjugate.



Scheme 4. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$ (5.0 equiv), CH_2Cl_2, 0 °C, 53%; (b) OsO_4 (0.01 equiv), NMO (1.1 equiv), acetone/H_2O 1.4:1, 90%; (c) acetic anhydride (1.1 equiv), DMAP 0.1 equiv), Et_3N (2.0 equiv), CH_2Cl_2, 0 °C, 65%; (d) DAST (1.2 equiv), CH_2Cl_2, −78 °C, 25%; (e) **46** (2.0 equiv), 2 M Na_2CO_3 (4.0 equiv), $PdCl_2$dppf (5 mol %), DMF, 80 °C, 44%; (f) K_2CO_3 (1.5 equiv), MeOH, 65%; (g) dog liver microsomes incubation, 37 °C, 3.5 h, SPE purification.

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25. Compounds **12D**, **13D** and **14** were stable in plasma over a 24–h period: no deuterium loss or reduction observed, respectively.
26. MK-0674 was incubated at 100 μM final concentration in phosphate buffer (60 mM, pH 7.4) containing 1 mg/mL of dog liver microsomes, 12.5 mM $MgCl_2$, 21 mM D-saccharic acid 1–4 lactone and 13 mM of UDPGA for 3.5 h at 37 °C under 95/5 O_2:CO_2 atmosphere. Incubation broth (~80 mL) was centrifuged 5 min at 4300 rcf, the supernatant collected, and then the pellet was washed with 1 mL of acetonitrile. Aqueous and organic fractions were combined and an Oasis HLB 6 cc cartridge from Waters was used to isolate the glucuronide conjugate of MK-0674.
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