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Difluoroketones as Inhibitors of Matrix Metalloprotease-13

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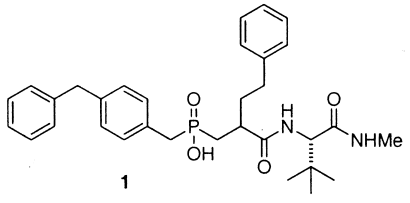
Abstract—Substrate-like difluoroketones have been prepared as potential inhibitors of MMP-13. Weak inhibition was seen with the key target **2**. This and the more potent activity of intermediate **7b** illustrates that hydrated ketones can be used to inhibit MMP-13 and perhaps other members of this class of enzymes.

The inhibition of matrix metalloproteases (MMP's) has been effected with compounds containing a variety of zinc binding groups including hydroxamic acids, carboxylic acids, thiols and phosphinic acids.[1–4] While potent compounds containing each of these zinc ligands have been described, each ligand class possesses properties that have, in general, hampered their development into therapeutic agents. As a result, we have continued to examine other possible zinc ligands. In this regard, we noted that α-fluorinated ketones,[5] binding in their hydrated form, have been shown to be inhibitors of metalloproteases such as carboxypeptidase A,[6–8] angiotensin converting enzyme,[6] metallo-β-lactamase,[9] and membrane-bound amino peptidase.[10] Being transition state mimics, α-fluorinated ketones can readily bind in a substrate-like fashion in pockets on both side of an enzyme's catalytic residues. In this respect α-fluorinated ketones resemble phosphinic acid-based inhibitors. That they are both monodentate ligands for the active site zinc is another similarity observed in the available crystal structures;[1,7,11,12] however, a significant difference between them is their pK_a's. Phosphinic acids will be nearly completely deprotonated at physiological pH (pK_a's ~3)[13] and hydrated α-fluorinated ketones will be substantially uncharged (trifluoroacetone pK_a = 10.2).[14] This difference may translate into better absorption for a α-fluorinated ketone-based inhibitor over a phosphinate-based compound of otherwise similar structure. Orally active α-fluorinated ketone-based protease inhibitors have been described.[15] Owing to their not being deprotonated at physiological pH, α-fluorinated ketone-based inhibitors may also have an inherent potency advantage over phosphinates in that upon binding neither the ligand nor the active site glutamate needs to be protonated by bulk water in order to avoid an unfavorable charge-charge interaction.[1]

The above aspects of α-fluorinated ketone-based inhibitors combined with our recent finding that phosphinate **1** is a potent inhibitor of MMP-13 (IC_{50}: 14 nM)[11] led us to prepare the difluoroketone analogue **2** as a probe to examine the utility of this class of compounds for the inhibition of MMP's. Difluoroketone **2** contains functionality suitable for binding into the S_2, S_1', and S_3' pockets of MMP-13.[11] We chose to prepare **2** over the isomeric α,α-difluoroketone **3** since the latter may be unstable due to β-elimination of HF.[16–18]



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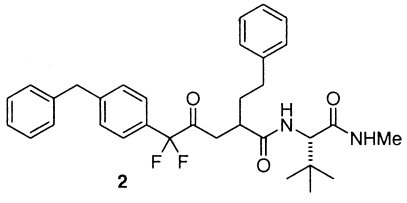

2

In order to determine the best methods for approaching the synthesis of **2**, as well as to facilitate the interpretation of NMR spectra through the elimination of diastereomers, our initial target was **4**, the derivative without the phenethyl side chain. The successful synthetic route parallels that of Hong[18] in that the P_1' carboxylate is released through the oxidation of a terminal olefin.

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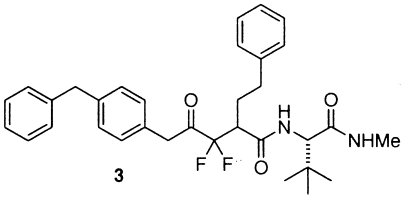
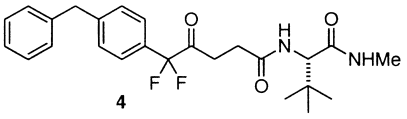
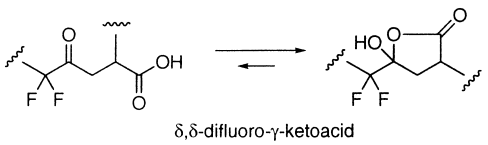
Accordingly, the α-ketoester **5**[19] was treated with DAST[20] and the resultant α,α-difluoroester converted in three standard steps to the Weinreb amide **6** (see Scheme 1). This was treated with 3-butenylmagnesium bromide giving the desired ketone **7a**. In an earlier unsuccessful route, we found that production of a δ,δ-difluoro-γ-keto acid led to the formation of a stable cyclic derivative which we could not induce to react in its open-chain carboxylate form.[21] Thus, we did not wish to release the terminal carboxylic acid until after the ketone was appropriately protected. Since protection of this highly electron-deficient ketone as a ketal was not expected to be facile,[22,23] we reduced **7a** to the alcohol **8a** and protected this as its acetate. Ruthenium catalyzed cleavage of the olefin[24] gave the expected carboxylic acid **9a**. Coupling of the carboxylate with *N*-methyl-*tert*-leucine and deacetylation gave the penultimate alcohol **10a**, oxidation of which with Dess–Martin reagent[25] yielded the desired α,α-difluoroketone **4**.

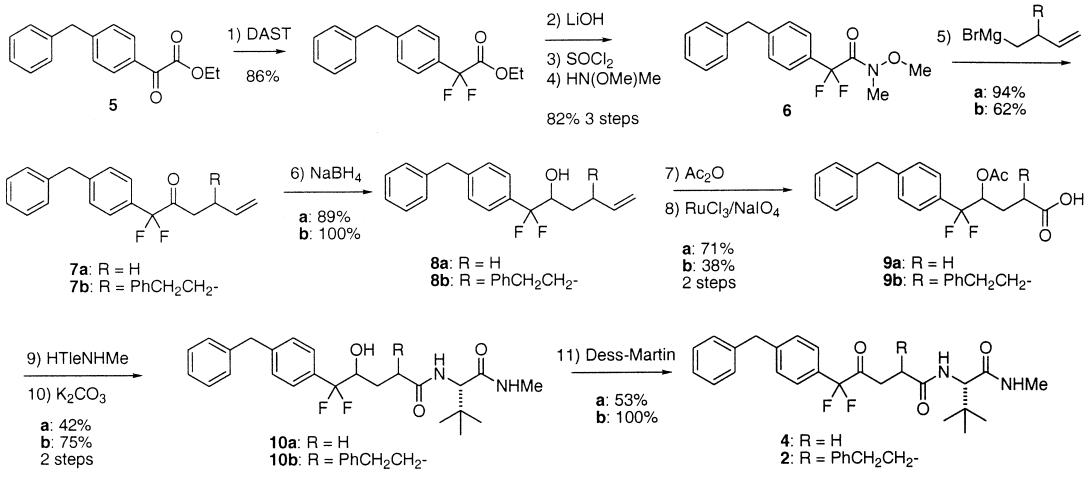




In order to prepare **2**, a Grignard reagent corresponding to 3-butenylmagnesium bromide but containing a 2-phenethyl side chain was required. This was prepared in two steps: a S_N2' reaction of 1,4-dibromo-2-butene with phenethylmagnesium chloride[26] in the presence of CuCN·2LiCl[27] followed by Grignard formation using Rieke magnesium.[28] This Grignard reagent and **6** yielded the expected ketone **7b**. As before, reduction gave the alcohol **8b** which was acetylated, oxidized, coupled to *N*-methyl-*tert*-leucine and deacetylated yielding alcohol **10b**. Oxidation gave the target compound **2**.

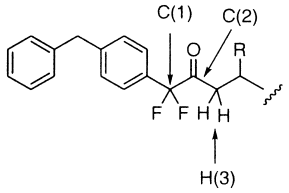


NMR spectroscopy was used to demonstrate that the target compounds hydrated in the presence of water and did not exist as hydroxy lactams.[29] Thus, the ^{19}F NMR of **7a** in dry DMSO-d_6 shows a singlet at −105.1 ppm (Table 1). Dilution with D_2O results in the appearance of another signal in the ^{19}F NMR spectrum at −108.8 ppm. This shift is consistent with that expected for hydration of a fluoroketone.[30,31] The formation of the hydrate was further confirmed through the ^{13}C and ^{1}H NMR spectra. In dry DMSO the carbonyl carbon C(2) was observed at 198.6 ppm, the fluorine substituted carbon C(1) at 115.4 ppm, and the protons H(3) on the carbon adjacent to the ketone at 2.80 ppm. In the presence of D_2O additional signals appeared at 94.1 ppm for C(2), at 120.7 ppm for C(1), and at 1.52 ppm for the methylene group, all of which are consistent with hydration of the ketone. The percent hydration observed after 24 h was determined by integration of the ^{19}F and ^{1}H NMR signals and was on the order of 27%. The NMR spectra of **4** under the same conditions revealed corresponding shifts in the signals for the fluorine, carbon and hydrogen atoms. Hydration after 24 h was determined to be 42%. That similar shifts were observed for **7a** and **4** indicates that hydroxy lactam formation by cyclization of the secondary amide of **4** onto the ketone moiety was not occurring to any significant degree. This point was reinforced by the ^{19}F NMR of the tertiary amide **11**[32] (see Table 2 for structure) which, like **7a**, is incapable of forming a cyclic structure and which shows a comparable shift between the ^{19}F resonances for the ketone and the hydrate. Since **2** was a mixture of diastereomers the ^{1}H and ^{13}C NMR spectra were too complex to be interpretable at the same level of detail; however, the ^{19}F NMR showed parallel shifts of the diastereotopic fluorine atoms for both diastereomers. While the percent hydration of **2** was only 4% after 24 h,[33] in a higher concentration



Scheme 1. Synthesis of difluoroketones **2** and **4**. Reaction conditions. (i) Neat, rt, 18 h; (ii) 10% aq ethanol, rt 18 h; (iii) cat. DMF, CH_2Cl_2, rt, 2 h; (iv) DIEA, CH_2Cl_2, rt, 18 h; (v) THF, 0 °C to rt, 18 h; (vi) ethanol, rt, 1 h; (vii) pyridine, rt, 60 h; (viii) $CCl_4/CH_3CN/H_2O$, rt, 2 h; (ix) BOP, CH_2Cl_2, rt, 18 h; (x) MeOH, rt, 2 h; (xi) CH_2Cl_2, rt, 2 h. All compounds were characterized by ^{1}H and ^{19}F NMR and MS and purity assesed by reverse-phase HPLC.

Table 1. Selected NMR data for difluoroketones **2**, **4**, **7a**, and **11**



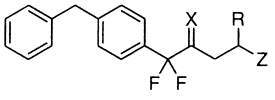
Compound	Solvent[a]	^{19}F[b,c]		$^{13}C(1)$[c]	$^{13}C(2)$[c]	$^{1}H(3)$[c]
7a	Dry DMSO	−105.1 s		115.4 t (J=254)	198.6 t (J=30.3)	2.80 m
	Wet DMSO	−108.8 (28%)[d]		120.7 t (J=252)	94.1 t (J=30.3)	1.52 (26%)[b]
4	Dry DMSO	−103.8 s		115.3 t (J=252)	198.7 t (J=31.8)	2.89 m
	Wet DMSO	−108.8 (42%)[d]		120.5 t (J=253)	93.7 t (J=29.2)	1.69 (39%)[b]
11	Dry DMSO	−103.6/−104.3 2d (J=251)		—	—	—
	Wet DMSO	−108.7/−109.0 (23%)[d]		—	—	—
2	Dry DMSO	−103.5/−104.4 2d (J=252)	−103.9 s	—	—	—
	Wet DMSO	−108.90/−108.93 (4%)[d]	−108.8 s	—	—	—

[a]Dry DMSO: sample dissolved in 1.0 mL DMSO-d_6 (from an ampoule) in an oven dried NMR tube; wet DMSO: above sample diluted with 0.10 mL D_2O.
[b]ppm relative to $CFCl_3$.
[c]s, singlet, d, doublet, t, triplet, m, multiplet.
[d]Percentage of hydrate present 24 h after dilution of the sample.

of water (0.20 mL D_2O in 1.0 mL DMSO-d_6) and after 48 h, 14% of the material existed in its hydrated form.

Evaluation of the target ketones **2** and **4** as well as some of the intermediates revealed them to be, in general, weak inhibitors of MMP-13 (Table 2).[34] The key target **2** has an IC_{50} of 5 μM, more than 100-fold higher than phosphinate **1**. Despite this relatively weak inhibition, the difluoroketone moiety does appear to be critical to the observed inhibition. Thus, **10b**, the alcohol precursor of **2**, has an IC_{50} greater than 30 μM. Even more telling in this regard is the comparison of **7b** and **8b**. Ketone **7b** is the most potent compound in the series having a sub-μM IC_{50} while the corresponding alcohol is nearly 100-fold less active. Although the hydrated difluoroketone moiety may be acting as a zinc ligand as desired, a comparison of the differences in activity between **2**, **4**, and **11** suggests that the compounds are not binding to the enzyme in the expected substrate-like manner. In other series of substrate-like MMP inhibitors, removing the key P_1' side chain leads to a large reduction in binding affinity.[1] This appears to be reflected in the difference between **7a** and **7b**; however, in the case of the amide-containing analogues **2** and **4**, removal of the P_1' side chain leads to only a ~2-fold decrease in activity. Truncation of the P_2'–P_3' region also typically leads to substantial potency reduction.[1] Such a potency change is not observed in the present case as **2** and **11** are essentially equi-active. The binding differences observed for **2**, **7b**, **11** and **12**[35] may derive from an interplay between the conformational and steric effects conferred by the substituent β to the ketone. Thus, the vinyl group of **7b** may drive the molecule into a favorable conformation and

Table 2. MMP-13 Activity of difluoroketones and intermediates



Compound	X	R	Z	MMP-13 IC_{50} (μM) (n)
7a	=O	H—	-CH=CH_2	>>30
4	=O	H—	-COTle[a]	12.0 + 4.2 (2)
7b	=O	$PhCH_2CH_2$–	-CH=CH_2	0.29 + 0.1 (2)
8b	-OH,-H	$PhCH_2CH_2$–	-CH=CH_2	27
10b	-OH,-H	$PhCH_2CH_2$–	-COTle[a]	>30
2	=O	$PhCH_2CH_2$–	-COTle[a]	5.1 + 3.8 (3)
11	=O	$PhCH_2CH_2$–	-CON$(CH_2)_5$	6.1 + 1.5 (2)
12	=O	$PhCH_2CH_2$–	-H	>30

[a]COTle:-*N*-carbonyl-*N*′-methyl-*tert*-leucine amide.

be small enough to escape a negative steric interaction with the enzyme upon binding. In contrast, the larger amide functionalities of **2** and **11** may drive these molecules into a favorable overall conformation but be too large to allow as efficient binding to the enzyme. Ketone **12**, which lacks a substituent β to the ketone, may simply not adopt a conformation favorable for binding.

The apparent failure of **2** and the other difluoroketones to bind in the expected manner could be due to a number of factors. For example, examination of **2** 'bound' into a model of MMP-13 in a substrate-like orientation reveals a potential negative dipole interaction between one of the fluorine atoms and the carbonyl of Ala-186, which are closer than a typical O-F van der Waals contact.[36] Another relevant factor may be the bond angle for the

C–CF_2–C group in **2** which is likely to be more obtuse[37] as compared to the corresponding C–CH_2–C of **1**, a change which will substantially alter the position of the terminal P_2 phenyl ring.

In conclusion, we have described the synthesis of a series of difluoroketones that were designed to be inhibitors of MMP-13. While the key target **2** was only a weak inhibitor of the enzyme, our hypothesis regarding the use of difluoroketones as inhibitors of MMP's is validated by the activity observed, especially the sub-μM activity of **7b**. Although the binding orientation of the active compounds does not appear to be as predicted, our results suggest that the potency may be improved by varying both of the groups β to the ketone. This and an assessment of any stereochemical bias imparted by these groups may lead to a better understanding of the binding mode of these compounds.

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32. Compound **11** was prepared by a sequence of reactions that parallels the synthesis of **2** but starting from **9b** and piperidine.
33. It has been proposed that the side chain of *tert*-leucine when incorporated into substrate-like MMP inhibitors shields the adjacent amides from hydration (refs 1, 11, and 43). The low hydration of **2** at 24 h as compared to **4**, **7b** and **11** under the same conditions may be a reflection of this phenomenon.
34. MMP-13 IC_{50}'s were determined as indicated in our earlier work (ref 11). Since $K_m >> [S]$, IC_{50}'s $\approx K_i$'s. Pre-incubation of **2** with enzyme for various time periods before addition of substrate did not lead to a significant change in its IC_{50} indicating that slow binding of the inhibitor was not an issue.
35. Compound **12** was prepared from **6** and phenbutylmagnesium bromide.
36. We thank Dr. E. R. Laird of Computational Chemistry, Central Research Division, Pfizer Inc for modeling **2** in an MMP-13 structure.
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