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SYNTHESIS OF THIOPHENOL DERIVATIVES AS INHIBITORS OF HUMAN COLLAGENASE

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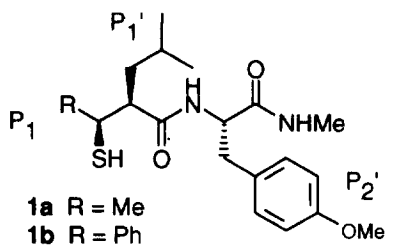
Abstract: A series of peptidomimetic thiophenol derivatives has been prepared and evaluated *in vitro* as inhibitors of human fibroblast collagenase. Many of these compounds have IC_{50} values in the sub-micromolar range.

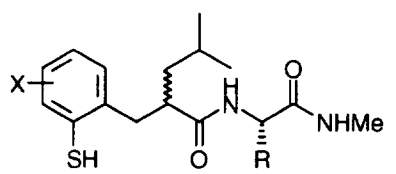
Collagenase (MMP-1) is a member of the family of zinc-containing matrix metalloproteinases (MMPs), and is thought to play a major role in the destruction of connective tissue components of articular cartilage.[1] The rational design of low molecular weight inhibitors of collagenase has been reviewed,[2,3] a key feature being the inclusion of a moiety capable of binding to the zinc of the enzyme active site.

The thiol group has been shown to be an effective zinc ligand in a number of potent inhibitors of collagenase.[4,5,6] However, in all cases, the thiol group is bonded to an aliphatic carbon. Since the chemical and physicochemical properties of aromatic thiols are known to differ from those of their aliphatic counterparts, it was of interest to determine whether collagenase inhibitory activity could be attained with molecules containing a thiophenol group. A similar approach proved successful for inhibitors of angiotensin converting enzyme (ACE), another zinc metalloproteinase.[7]

The aliphatic thiols **1a** and **1b** were reported[5] to have IC_{50} values of 0.22 and 0.27 μM respectively against collagenase, the latter indicating the tolerance of an aromatic ring at the P_1 position. The compounds described in this paper, **2**, have the thiol group transposed from its benzylic position in **1b** to an aromatic ring carbon. Modelling studies[8] indicated that low energy conformations of **2** are available that allow the thiophenol sulphur to approach the space assumed to be occupied by the active site zinc.



1a R = Me
1b R = Ph



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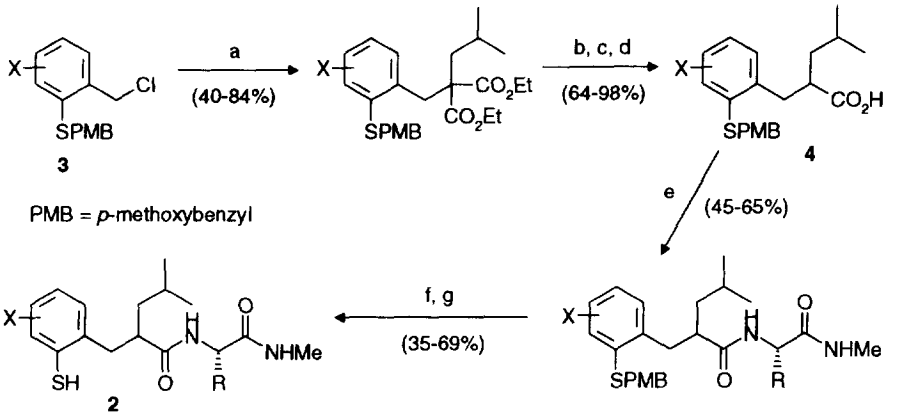
In the current work, residues known[3] to impart potent collagenase activity were incorporated into the P_1' and P_2' positions (the P_1' group was kept constant as *iso*-butyl), whilst substitution around the thiophenol ring was varied. The inhibitors were prepared as in Scheme 1. Substituted benzyl chlorides **3** were converted by malonic ester synthesis to the aryl propionic acid derivatives **4**. These were coupled to known P_2' (*S*)-α-amino acid derivatives and the sulphur protecting groups were removed with $Hg(OAc)_2$ in TFA.[9]

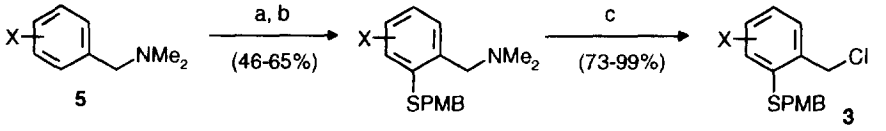
Scheme 1



Reagents: (a) i-BuCH(CO_2Et)$_2$, NaH, DMF, 100 °C, (b) 40% NaOH, EtOH, Δ, (c) 2N HCl, (d) xylene, Δ, (e) EDC, $H_2NCH(R)CONHMe$, CH_2Cl_2, (f) $Hg(OAc)_2$, TFA, anisole, (g) H_2S, DMF

A flexible synthesis (Scheme 2) was developed for chlorides **3** with a range of ring substituents, based on heteroatom assisted ring lithiation[10] of the dimethylaminomethyl arenes **5**, followed by reaction with *p*-methoxybenzyl disulphide. The dimethylamino group could then be converted to a chloride leaving group by ethyl chloroformate treatment.[11]

Scheme 2

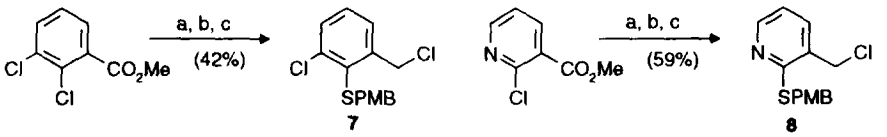


Reagents: (a) n-BuLi, Et_2O, (b) $(PMBS)_2$, THF, (c) EtO_2CCl, Et_2O

An exception was the ortho-chloro substituent **7**, which would have eliminated to a benzyne on lithiation, and was prepared instead by nucleophilic substitution of the dichloroester as shown in Scheme 3. The pyridine analogue **8** was prepared similarly.

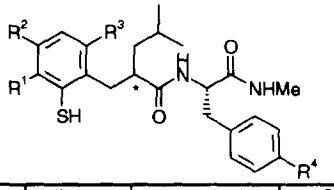
The thiophenols were assayed with β-mercaptoethanol added to the assay to minimise oxidation of the thiol group to disulphide, as described previously.[4] The results are summarised in Table 1.

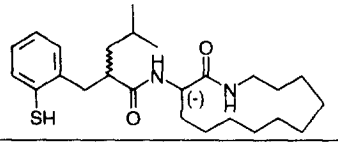
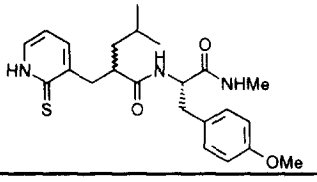
Scheme 3



Reagents: (a) PMBSH, NaH, DMF, 70 °C, (b) KBH_4, LiCl, THF, Δ, (c) MsCl, Et_3N, CH_2Cl_2, 18 h

Table 1. Potency of Thiophenol Derivatives as Collagenase Inhibitors



Entry	R^1	R^2	R^3	R^4	chirality *	$[\alpha]^{20}_D$ deg (MeOH)	IC_{50} μM[a]
1	H	H	H	MeO	S	+22	0.055 ± 0.014 (n=6)
2	H	H	H	MeO	R	-85	0.34 ± 0.06 (n=2)
3	MeO	H	H	MeO	S		0.16
4	MeO	H	H	MeO	R	-68	0.22
5	H	MeO	H	MeO	RS		0.14
6	Cl	H	H	MeO	RS		0.33
7	H	Cl	H	MeO	RS		0.23 ± 0.02 (n=3)
8	H	H	Cl	MeO	RS		0.32
9	CF_3	H	H	MeO	RS		0.29
10	H	H	H	H	S	+23	0.030 ± 0.002 (n=2)
11	H	H	H	H	R	-83	1.1
12					RS		>100
13					RS		>100
1a							0.017 ± 0.008 (n=2)

[a]Unless otherwise indicated, n=1. All assays included entry 1 as control.

The first compounds tested (entries 1 to 4) had (*S*)-Tyr(Me)NHMe in the P_2' position and were separated into single diastereoisomers prior to S-deprotection. The *S,S* isomer (entry 1) (stereochemistry

established by X-ray[12]) was found to be 6-fold more active than the *R,S* isomer (entry 2). Less differentiation in activities was seen for the ortho-methoxy diastereoisomers (entries 3 and 4). Subsequent compounds in this series were tested as unseparated mixtures of diastereoisomers.

The potency of the compounds was relatively unaffected by substitution around the thiophenol ring, with the electron donating methoxy substituents (entries 3 to 5) having similar potency to the electron withdrawing chloro and trifluoromethyl groups (entries 6 to 9). Similarly, the position of substitution had little effect on potency, suggesting that all the substituents studied are equally well accommodated in the S_1 binding pocket. This contrasts with the thiophenol inhibitors of ACE where ortho substitution was prefered.[7] The potency of the *S,S* diastereoisomer was enhanced (entry 10 versus 1) and the difference in activity between the diastereoisomers was increased (entries 10 and 11 versus 1 and 2) when (*S*)-PheNHMe was substituted at P_2'. Thus the most potent compounds of this series had activities approaching that of the aliphatic thiol **1a**.

Introduction of a 13-membered lactam, known to be an effective P_2'-P_3' substitution in other series of collagenase inhibitors,[2,13] caused a dramatic loss of potency (entry 12). This may be due to its highly lipophilic nature (clogP = 9; compare with entry 1, clogP = 4.4)[14] and consequent poor aqueous solubility. The pyridine thione (entry 13) was found to be inactive. The known predominance of the thione tautomer[15] over the thiol form suggests that a free sulphydryl group is necessary for binding.

The natural substrate and the majority of synthetic inhibitors of collagenase have the (*S*)-Leu absolute configuration at P_1'. It is of interest, therefore, that in this series the prefered stereochemistry at P_1' is *S*, which corresponds to the unnatural (*R*)-Leu configuration. A similar unexpected preference has been reported[6] for another series of thiol-based collagenase inhibitors.

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