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Structure-Based Design of Ketone-Containing, Tripeptidyl Human Rhinovirus 3C Protease Inhibitors

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Abstract—Tripeptide-derived molecules incorporating C-terminal ketone electrophiles were evaluated as reversible inhibitors of the cysteine-containing human rhinovirus 3C protease (3CP). An optimized example of such compounds displayed potent 3CP inhibition activity ($K_i = 0.0045$ μM) and in vitro antiviral properties ($EC_{50} = 0.34$ μM) when tested against HRV serotype-14.

The human rhinoviruses (HRVs) are members of the picornavirus family and are the single most significant cause of the common cold.[1,2] The replication of these viruses requires the proteolytic processing of a large polyprotein produced by cellular translation of the viral RNA genome. This processing is primarily accomplished by the human rhinovirus 3C protease (3CP),[3,4] a cysteine protease possessing minimal homology with prevalent mammalian enzymes but which exhibits structural similarity to the trypsin protein family.[5] As a critical component of the rhinovirus replication cycle, the 3C protease is a potential target for the development of novel antirhinoviral agents. Several examples of reversible and irreversible 3CP inhibitors have recently appeared in the literature, and some of these entities display in vitro antiviral properties.[6] Substrate-derived[3,4,7] peptide aldehydes, typified by compound **1**[8] ($K_i = 0.006$ μM, $EC_{50} = 2.4$ μM), were among the first reversible 3CP inhibitors so described.[8–12] These molecules form covalent hemithioacetal adducts with the active site 3CP cysteine residue[8] in a manner analogous to the interaction of related peptide aldehydes with various serine proteases.[13] However, concern over the possible biological liabilities associated with the aldehyde functional group (e.g. toxicity, selectivity and stability) prompted us to identify reversible 3C protease inhibitors which contained alternate electrophiles. In this report, we describe the exploration of ketone-containing molecules, some of which display potent 3CP inhibition activity and antirhinoviral properties.

Replacement of the aldehyde group present in **1** with a 2-benzothiazole ketone moiety afforded an active, albeit significantly less potent, reversible 3CP inhibitor **2** (Table 1). The combination of related ketone electrophiles with appropriate peptidyl entities has also provided reversible inhibitors of several other cysteine[14–16] and serine[17–22] proteases. Unfortunately, compound **2** did not display in vitro antirhinoviral activity when tested to concentrations at which cytotoxicity (CC_{50}) was observed. However, replacement of the P_1 glutamine isostere of **2** with the natural Gln amino acid side-chain afforded a compound **3**, which exhibited reduced 3CP inhibition activity but *improved* antiviral properties with less observed cytotoxicity. Importantly, reduction of the ketone electrophile contained in **3** to the corresponding alcohol **4** resulted in considerable loss in anti-3CP activity. This result was consistent with the formation of a covalent hemithioketal inhibitor-enzyme adduct through addition of the 3CP active site cysteine residue to the ketone moiety of **3**. Introduction of a P_1-lactam

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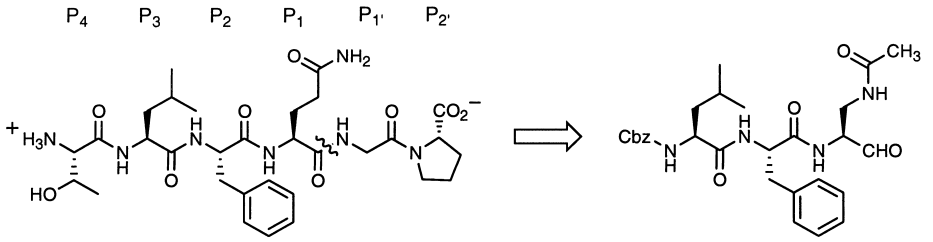
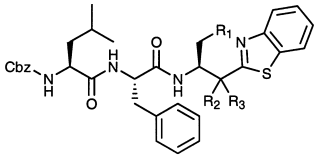


glutamine replacement into the inhibitor design (compound **5**) greatly improved 3CP inhibition activity relative to **3** in a manner anticipated from studies of related Michael acceptor-containing 3CP inhibitors.[23,24] Curiously, compound **5** displayed only moderately improved in vitro antirhinoviral properties relative to **3**. As was observed for compound **3** above, reduction of **5** to the corresponding alcohol **6** resulted in significant loss of anti-3CP activity and supported the formation of a 3CP-**5** hemithioketal adduct.

Having determined that tripeptidyl molecules containing P_1-lactam and C-terminal 2-benzothiazole ketone moieties could function as potent, reversible 3CP inhibitors, we then examined modification of the ketone electrophile. Incorporation of a 2-thiazole into the inhibitor design resulted in significant loss of anti-3CP activity and a somewhat lesser reduction in antiviral potency (compare **7** with **5**, Table 2). In contrast, replacement of the 2-benzothiazole moiety present in **5** with a 2-pyridine only slightly diminished 3CP inhibition properties (compound **8**). However, a molecule containing a 2-benzothiophene electrophile displayed drastically reduced anti-3CP activity and showed no measurable antiviral properties when tested to the 10 μM level (compare **9** with **5**). This result paralleled those obtained with related serine protease inhibitors and was consistent with the formation of a hydrogen bond between the benzothiazole nitrogen atom and a protonated His residue in the 3CP active site.[20,22] Subsequent analysis of the 3CP-**5** X-ray crystal structure confirmed such an interaction as well as the formation of an inhibitor enzyme hemithioketal adduct.[26] A similar reduction in anti-3CP activity was exhibited by a compound which incorporated a phenyl ketone electrophile in lieu of a 2-pyridyl ketone moiety (compare **10** with **8**, Table 2).

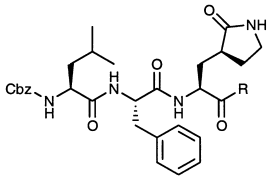
Table 1. [25]



Compd	R_1	R_2	R_3	K_i (μM)[a]	EC_{50} (μM)[a]	CC_{50} (μM)
2		=O		1.7	> 25	25
3		=O		3.5	17	> 100
4[b]		OH	H	NI[c]	32	80
5		=O		0.065	3.2	> 320
6[b]		OH	H	34	7.9	63

[a]Serotype-14.
[b]1:1 mixture of diastereomers.
[c]NI = no inhibition to 10 μM.

Table 2. [25]

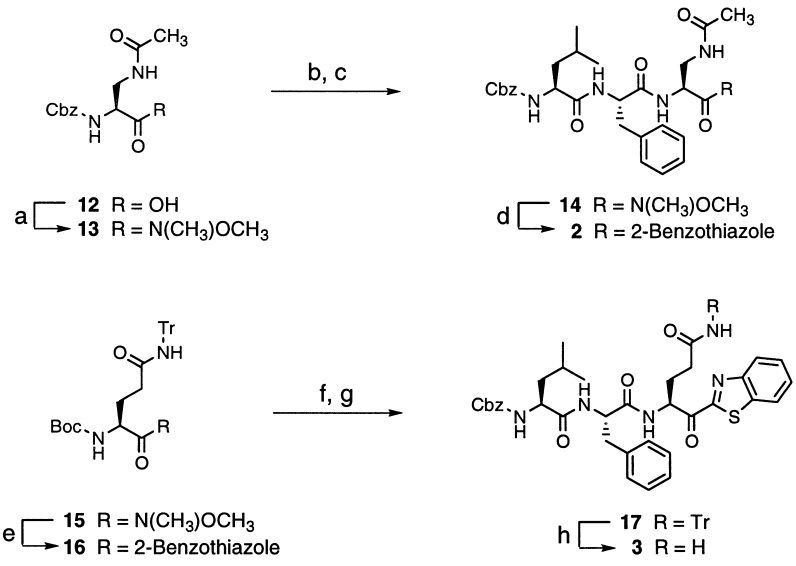


Compd	R	K_i (μM)[a]	EC_{50} (μM)[a]	CC_{50} (μM)
5		0.065	3.2	> 320
7		0.70	7.9	240
8		0.17	4.0	200
9		4.7	> 10	> 10
10		3.2	> 10	> 10

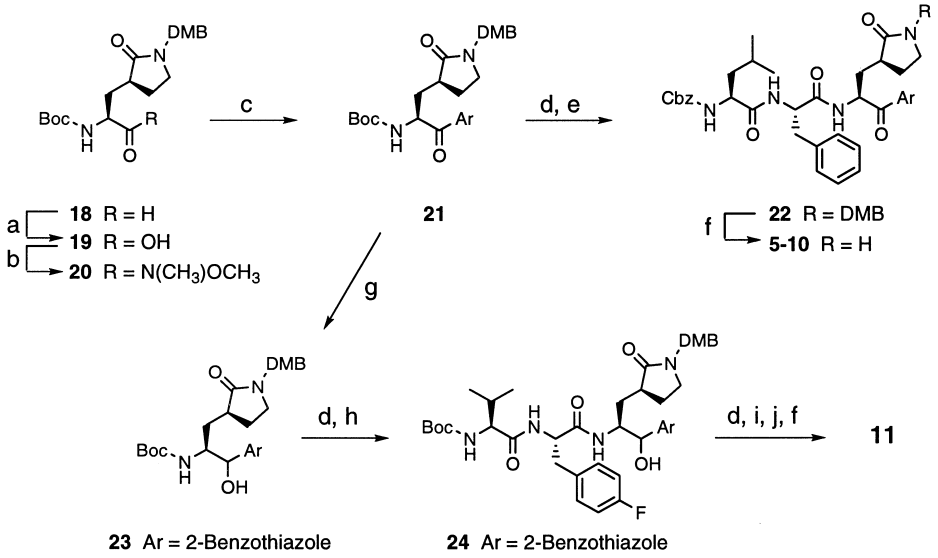
[a]Serotype-14.

The structure–activity results illustrated in Table 2 identified the 2-benzothiazole as an ideal fragment for incorporation into C-terminal ketone-containing 3CP inhibitors. Accordingly, this moiety was combined with a tripeptidyl binding element that was previously shown to impart high levels of anti-3CP activity to related Michael acceptor-containing molecules.[23] The resulting compound **11** displayed very potent levels of reversible 3CP inhibition along with sub-micromolar antiviral activity against several rhinovirus serotypes. In addition, the molecule did not exhibit significant cytotoxicity when tested in cell culture. Compound **11** therefore, ranks as one of the most potent reversible 3CP inhibitors reported to date and its antirhinoviral activity compares quite favorably with that displayed by related peptide aldehydes.[8–11]

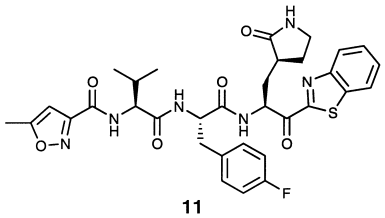
As illustrated in Schemes 1 and 2, the 3CP inhibitors described in this work were prepared by methods related to those utilized previously to synthesize tripeptidyl Michael acceptor-containing compounds.[23,27] In general, an appropriate organolithium species was condensed with an amino acid or peptide-derived Weinreb amide[28] entity to form the desired ketone electrophiles. For the preparation of compound **11**, the initially formed ketone moiety (**21**, Ar = 2-benzothiazole) was reduced to the corresponding alcohol **23** prior to peptide coupling in order to minimize racemization of the



Scheme 1. Reagents and conditions (Tr = CPh_3): (a) EDC, HCl·$HN(CH_3)OCH_3$, NMM, CH_2Cl, 23 °C, 18 h, 78%; (b) H_2, Pd on C, CH_3OH, 23 °C, 2 h; (c) Cbz-L-Leu-L-Phe-OH, *N*-hydroxysuccinimide, DCC, CH_2Cl_2, 23 °C, 12 h, 89%; (d) 10 equiv 2-lithio-benziothiazole, THF, −78 to 23 °C, 18 h, 21%; (e) 10 equiv 2-lithio-benzothiazole, THF, −78 °C, 2 h, 35%; (f) HCl, 1,4-dioxane, 23 °C, 3 h; (g) Cbz-L-Leu-L-Phe-OH, HATU, DIEA, DMF, 0 °C, 45 min, 74%; (h) TFA, CH_2Cl_2, 23 °C, 49%.



Scheme 2. Reagents and conditions (Ar = see Table 2, DMB = 2,4-dimethoxybenzyl): (a) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH, 23 °C, 18 h, 66%; (b) isobutyl chloroformate, NMM, HCl·$HN(CH_3)OCH_3$, CH_2Cl_2, −20 to 23 °C, 2 h, 71%; (c) 3–10 equiv ArLi, THF, −78 °C, 2–4 h, 20–80%; (d) HCl, 1,4-dioxane, 23 °C, 3 h; (e) Cbz-L-Leu-L-Phe-OH, HATU, DIEA, DMF, 0 °C, 45 min, 37%; (f) DDQ, CH_3CN, 60 °C, 5–8 h, 50–80%; (g) $NaBH_4$, EtOH, 0 °C, 68%; (h) Boc-L-Val-L-Phe(4-F)-OH, HATU, NMM, CH_3CN, 0 °C, 45 min, 94%; (i) 5-methylisoxazole-3-carbonyl chloride, 2,4,6-collidine, CH_2Cl_2, 0 °C, 30 min, 65%; (j) Dess–Martin peroiodinane, CH_2Cl_2, 23 °C, 2 h, 94%.



11

	HRV-14	HRV-1A	HRV-10
K_i (μM)[a]	0.0045	ND	ND
EC_{50} (μM)	0.34	0.34	0.25
CC_{50} (μM)	250	250	250

[a]Inhibition of HRV 3C protease; ND = not determined.

P_1 side-chain.[29] The alcohol functionality was later oxidized to afford the desired product **11**.[30]

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30. In several instances, the DMB group was removed from intermediate **21** prior to conversion to final compounds **5**–**10**. Either synthetic sequence afforded the desired products in acceptable overall yields.