Michael Acceptors as Cysteine Protease Inhibitors

Maria M.M. Santos* and Rui Moreira

i-Med.UL, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Abstract: Cysteine proteases selectively catalyze the hydrolysis of peptide bonds. Uncontrolled, unregulated, or undesired proteolysis can lead to many disease states including emphysema, stroke, viral infections, cancer, Alzheimer's disease, inflammation, and arthritis. Cysteine proteases inhibitors thus have considerable potential utility for therapeutic intervention in a variety of disease states.

This review emphasizes on the new developments from literature reports on Michael acceptors as potential cysteine protease inhibitors, namely vinyl sulfones, α,β-unsaturated carbonyl derivatives and aza-peptides. These compounds irreversibly alkylate the active site cysteine residue *via* conjugate addition. Examples of Michael acceptors inhibitors that have already progressed to clinical testing are also presented.

Key Words: Cysteine protease inhibitors, vinyl sulfone, α,β-unsaturated carbonyl derivative, aza-peptide Michael acceptor, K-777, Ruprintivir.

INTRODUCTION

Cysteine proteases are proteolytic enzymes involved in the degradation of proteins. Based on their sequence homology, cysteine proteases are categorized in three structurally distinct groups, namely papain-like (clan CA), ICE-like (clan CD) and picornain-like (clan PA(C)) [1]. Clan CA and clan CD contain the majority of cysteine proteases. Clan CA includes cathepsins, calpains, and parasite cysteine proteases. Clan CD contains several important enzymes including caspases, legumain, clostripain, gingipains, and separase. The clan PA(C) includes the human rhinoviruses (HRVs) which are the single most significant cause of the common cold. Specifically, the activity of HRV 3C protease (3CP) is essential for viral replication.

Cysteine proteases have been implicated in very diverse disease processes ranging from cardiovascular, inflammatory, neurological, respiratory, viral, musculoskeletal, immunological, CNS disorders, to cancer [2]. In addition, cysteine proteases are also essential for the life cycle of several parasites: cruzain, from *Trypanosoma cruzi*, the ethiologic agent of Chagas' disease [3]; falcipain, from *Plasmodium falciparium* parasite, the causing agent of malaria [4]; and rhodesain, from *T. brucei*, the causing agent of sleep sickness [5]. Cysteine protease inhibitors thus have considerable potential utility for therapeutic intervention in a variety of disease states.

Many cysteine protease inhibitors have been designed using the recognition sequence of peptide substrates. However, their peptide content turns these inhibitors susceptible to amide bond cleavage by other proteases, leading to low oral bioavailability and poor pharmacological profiles. Thus, the design and development of new inhibitors should reduce the peptide character in order to improve membrane permeability, increase plasma half-live, reduce elimination, and improve oral availability, while maintaining high selectivity toward the protease target. Several inhibitors of cysteine proteases have been described on literature reports and some of them are already in different phases of clinical testing. Some examples of warheads used for cysteine protease inhibitors are substituted methyl ketones, aldehydes, epoxides and aziridines [6].

One type of cysteine proteases inhibitors that has received special attention in the last few years are those based on Michael acceptor scaffolds. This review emphasizes on the new development from the literature reports in the exploration of Michael acceptors as potential cysteine protease inhibitors and the corresponding structure-activity relationships. We will only shortly summarize some aspects about other type of cysteine protease inhibitors, since they are very well described in reviews recently published [2, 6-10].

MICHAEL ACCEPTORS INHIBITORS

Peptide and amino acid derivatives that contain a Michael acceptor are specific irreversible cysteine protease inhibitors. This class of inhibitors includes vinyl sulfones, α,β-unsaturated carbonyl derivatives, and miscellaneous derivatives (Fig. **1**).

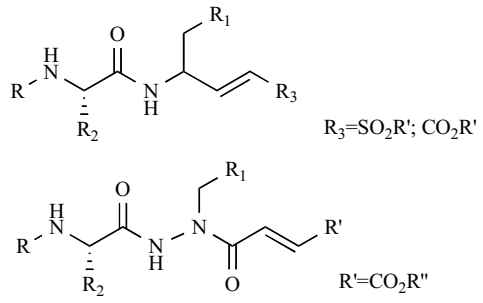

Fig. (1). Structure of peptide and aza-peptide Michael acceptors.

The mechanism of inhibition of cysteine proteases by Michael acceptors inhibitors proceeds *via* a Michael addition

*Address correspondence to this author at i-Med.UL, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-019 Lisboa, Portugal; Tel: +351 21 7946477; Fax: +351 21 7946470;
E-mail: mariasantos@ff.ul.pt

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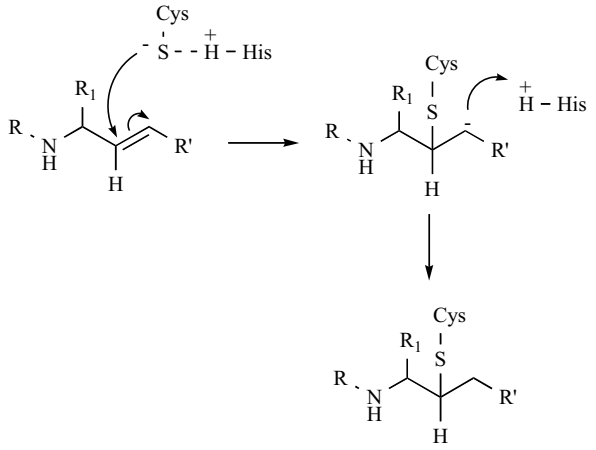
with an attack on the β-carbon by the active site cysteine residue followed by protonation of the α-carbon to form the thioether derivative (Scheme **1**).



Scheme 1. Mechanism of inhibition of cysteine proteases by Michael acceptors inhibitors.

One of the first Michael acceptors described in the literature was the fumarate derivative of E-64c (**1**), which was found to be an irreversible inhibitor of cathepsins B, H, and L (Fig. **2**) [11].

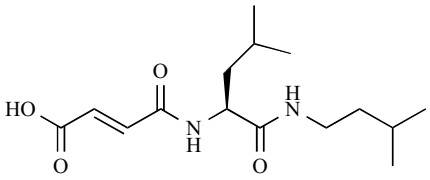
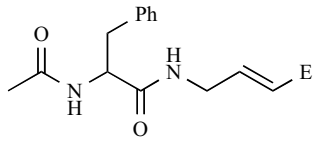


Fig. (2). Fumarate derivative of E-64c (**1**).

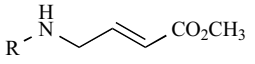
This review on Michael acceptors inhibitors will be subdivided into the three clans of cysteine proteases.

INHIBITORS FOR CLAN CA

Peptidic substrates in which the scissile amide carbonyl is replaced by a Michael acceptor were first introduced by Hanzlik and co-workers as specific irreversible inhibitors of the cysteine protease papain (Fig. **3**). Two series of peptidyl Michael acceptors were designed, the first of which varied the chemical reactivity of the Michael acceptor moiety (**2a-f**) while keeping the "recognition and binding" portion of the molecule constant; in the other series the "recognition and binding" portion of the molecule was varied (**3a-f**) keeping the chemical reactivity of the Michael acceptor moiety constant [12, 13]. In the first series the second-order rate constants obtained clearly showed that the electron-withdrawing ability of the E group does affect their relative inhibition activity toward papain and that there are no significant differences in binding. Moreover, compound (**2a**) showed time- and inhibitor concentration-dependent enzyme inactivation with a second-order rate constant of 70 $M^{-1}s^{-1}$. The Michael acceptor moiety proved to be essential for the inhibition, as the saturated and decarboxylated derivatives of (**2a**) showed only weak competitive inhibition [12, 13]. In the second series (**3a-f**), it was also shown that reducing the size of the P_2 side chains makes them less hydrophobic and less able to reach fully into the S_2 hydrophobic pocket, leading to significant decreases in inhibitor potency (Fig. **3**) [14].



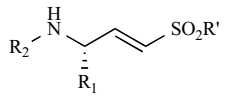
2a E=CO_2CH_3; **2b** E=SO_2CH_3; **2c** E=COOH;
2d E=CN; **2e** E=$CONH_2$; **2f** E=p-$O_2NC_6H_4$



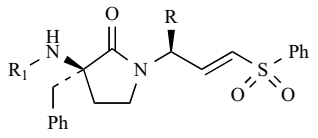
3a R=N-Ac-L-Phe; **3b** R=N-Ac-D-Phe; **3c** R=N-Ac-L-Leu;
3d R=N-Ac-L-Met; **3e** R=$PhCH_2CH_2CO$; **3f** R=PhCO

Fig. (3). Peptide Michael Acceptors.

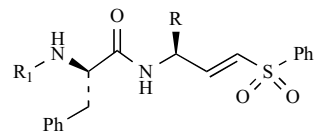
Potent vinyl sulfone inhibitors against disease associated cysteine proteases, such as cathepsins B, L, S, and O2, calpains, and cruzain were designed by Palmer and co-workers. They constructed peptidomimetic structures containing "functional scaffolds" enabling both inhibitor-protease binding and mechanistic inactivation (Fig. **4**). This family of inhibitors was most selective for cathepsin S, followed by cathepsin L, cathepsin O2, and cathepsin B. Cruzain was inhibited with similar efficiency to that of cathepsin L and the calpains were resistant to most inhibitors in this series. Nevertheless, the incorporation of known binding determinants for calpain (Leu-Leu-Tyr or Leu-Leu-Met) into a series of vinyl sulfones resulted in significant inactivation (**4i-m**) [15].



4a R_2=MuPhe; R_1=Hph; R'=Me
4b R_2=MuPhe; R_1=Hph; R'=Ph
4c R_2=MuTyr(3,5-I_2); R_1=Hph; R'=Ph
4d R_2=MuPhe; R_1=Lys; R'=PhHBr
4e R_2=MuLeu; R_1=Hph; R'=Ph
4f R_2=MuNp2; R_1=Hph; R'=2Np
4g R_2=MuPhe; R_1=Hph; R'=EtPh
4h R_2=Mu2Np; R_1=Hph; R'=EtPh
4i R_2=AcLeuLeu; R_1=Me; R'=Ph
4j R_2=AcLeuLeu; R_1=Nle; R'=Ph
4k R_2=MuLeuLeu; R_1=Tyr; R'=Ph
4l R_2=AcLeuLeu; R_1=Met(O_2); R'=Ph
4m R_2=ZLeuLeu; R_1=Tyr; R'=Ph



10a R=Me; R_1=Cbz
10b R=CH_2CH_2Ph; R_1=Cbz
10c R=CH_2CH_2Ph; R_1=SO_2Ph



11a R=Me; R_1=Cbz
11b R=CH_2CH_2Ph; R_1=Cbz
11c R=CH_2CH_2Ph; R_1=SO_2Ph

Fig. (4). Vinyl sulfone inhibitors.

To compare the S_2P_2 specificity of human cathepsins O2, S and L a series of compounds with the general structure

Mu-X-Hph-CH=CH-SO_2-Ph (X = Gly, Ala, Val, Leu, Ile, Ahx, Met, $MetO_2$, Phe) were synthesized. Cathepsin S was most efficiently inhibited displaying second-order rate constants as high as 10^7 $M^{-1}s^{-1}$. Cathepsins O2 and L were inhibited approximately one to three orders of magnitude less than cathepsin S. The S_2P_2 specificity for cathepsins O2 and L was similar, which may reflect similarities in the S_2 subsite architecture. The results indicate that the binding pocket of cathepsin S is deeper and more spacious than those of cathepsins O2 and L [16].

Rosenthal and co-workers evaluated these vinyl sulfones as potential falcipain inhibitors and thus as antimalarial agents. A number of vinyl sulfones inhibited falcipain-2 and three vinyl sulfones blocked parasite hemoglobin degradation and development at nanomolar concentrations [17]. Then, *N*-methylpiperazine-Leu-HPh vinyl sulfone (**5**) (Fig. **5**) was synthesized, and administered orally to mice. It markedly delayed the progression of murine malaria and cured about 40% of treated animals. This inhibitor showed improved activity compared to the phenyl vinyl sulfone counterpart, both in terms of the inhibition of falcipain and the inhibition of biological activities of cultured parasites [18].

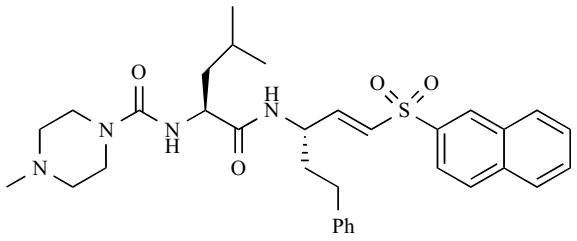


Fig. (5). Falcipain inhibitor (**5**).

Nevertheless, K777 (*N*-methylpiperazine-Phe-HPh-vinylsulfone) (**6**) (Fig. **6**), a Michael acceptor developed by Celera, is a potent inhibitor of cysteine proteases such as cathepsin B and L as well as cruzain [19].

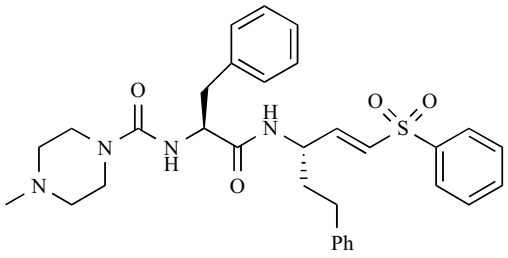


Fig. (6). Cruzain inhibitor K-777.

K777 has been shown to cure *T. cruzi* infection both in cell culture screen and in a mouse model of Chagas's disease [20]. It also protected beagle dogs from cardiac damage during infection by *T. cruzi* [21]. This inhibitor is orally bioavailable, and toxicology studies to date indicate that it is significantly safer than current therapy [22]. For these reasons, it has entered in clinical trials.

A series of vinyl sulfonamides and sulfonates inhibitors (Fig. **7**) were screened against cruzain. *N*-alkyl vinyl sulfonamides proved to be relatively weak inhibitors (**7b-e**). On the other hand, the simple sulfonate ethyl esters (**7a**) and (**8a**) proved to be much more potent. Phenyl vinyl sulfonate (**7f**) is an extremely potent inhibitor of cruzain. Comparative enzyme inhibition data for (**8b-d**) show that benzyl vinyl sulfone (**8c**) is the most potent of the three. These results indicated that a one-atom spacer between the sulfonyl unit and an aromatic ring is preferred, and that maximal activity is obtained when the spacer is an oxygen atom [23].

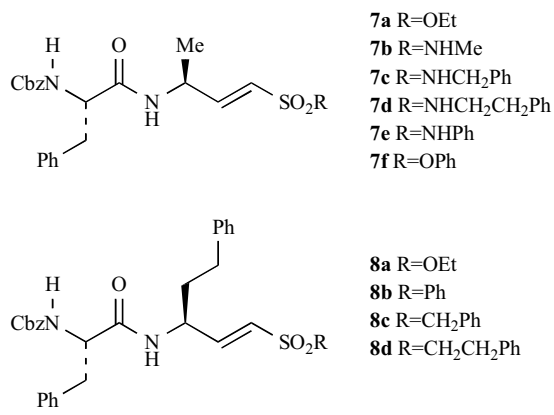


Fig. (7). Vinyl sulfonamides and sulfonate esters inhibitors.

Based on these results, a new family of potent *N*-alkoxyvinylsulfonamide inhibitors (Fig. **8**) of cruzain was developed. Although very potent cruzain inhibitors from vinyl sulfonate esters, vinyl sulfonamides and vinyl sulfone were examined, only one exhibited significant activity in the cell culture assay. Potent cruzain inhibitors with significant activity in the J774 macrophage cell culture assays, (**9a**) and (**9b**), were identified from several structures whose electronic properties at the sulfonyl group would be intermediate between those of the sulfonate esters and sulfonamides [24].

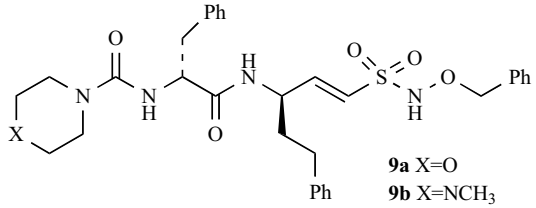


Fig. (8). *N*-alkoxyvinylsulfonamide inhibitors.

Structure-activity relationships for inhibition of falcipain-2, falcipain-3, and parasite development by vinyl sulfone, vinyl sulfonate ester, and vinyl sulfonamide cysteine protease inhibitors also suggested that peptidyl vinyl sulfones have potential as antimalarial agents. Levels of inhibition of falcipain-2 and falcipain-3 were generally similar. Considering SAR for enzyme inhibition, vinyl sulfonate esters, and vinyl sulfonamides offered improved potency over phenyl vinyl sulfones. Considering SAR for the inhibition of parasite development, the potency of inhibition of falcipain-2 and falcipain-3 was not an ideal predictor for activity against parasites. It was concluded that optimal antimalarial compounds, which inhibited *P. falciparium* development at low nM concentrations, contained P_2 leucine moieties [25].

Scheidt and co-workers synthesized inhibitors that contained a P_1-P_2 pyrrolidinone unit and a phenyl vinyl sulfone moiety (Fig. **4**). The conformationally constrained vinyl sulfones (**10a-c**) were significantly less active as inhibitors of cruzain when compared to the unconstrained analogues (**11a-c**). It was concluded that the added ethylene unit of the pyrrolidinone moiety must impose steric problems, preventing the inhibitors from having optimal binding in cruzain's active site [26].

Peptidyl vinyl sulfone as inhibitors of DPPI were also developed. The compounds contained either a phenyl or methyl group attached to the sulfone functional group. Val-Phe-VS-Ph, Val-Phe-VS-CH_3, and Gly-Phe-VS-CH_3 were very effective and nontoxic compounds. In particular, (**12**) (Fig. **9**) was the most effective non-toxic inhibitor and displayed selectivity versus cathepsin L [27].

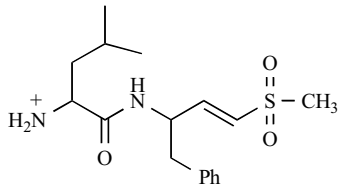


Fig. (9). Peptidyl vinyl sulfone inhibitor (**12**).

Pyrimidinyl peptidomimetic agents were synthesized, and their *in vitro* antimalarial activities against *P. falciparum* were evaluated. These new agents contained a Michael acceptor side chain methyl 2-hydroxymethyl-but-2-enoate, so that once the active site cysteine has added to the unsaturated ester, it can eliminate to form a new unsaturated ester covalently linked to enzyme. This new Michael acceptor can react with another active site nucleophile such as the His, or it may just stabilize the initial adduct. Compound (**13**) (Fig. **10**) displayed antimalarial efficacy comparable to that of chloroquine, but no falcipain inhibition data was disclosed [28].

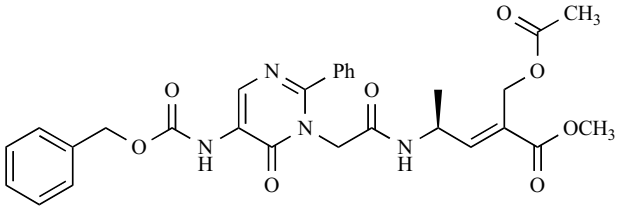
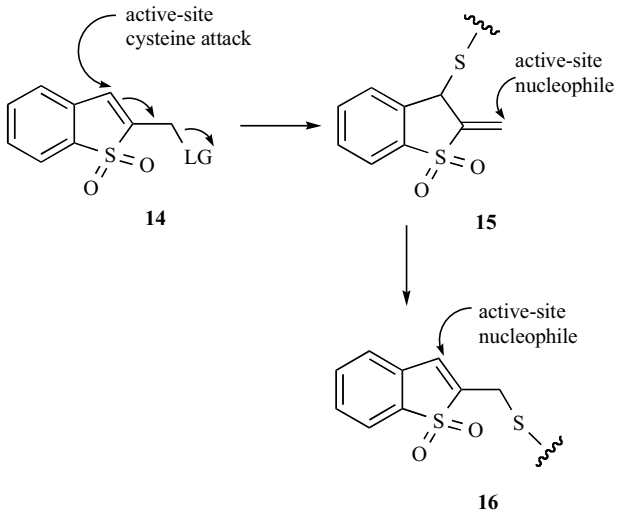


Fig. (10). Pyrimidinyl peptidomimetic inhibitor (**13**).

Recently, the Bsmoc amino-protecting group was suggested as a scaffold for double-hit inhibitors. Cyclic vinyl sulfones (**14**) derived from Bsmoc, were shown to be irreversible inhibitors of papain and cathepsin B. Compounds (**14**) with a good leaving group (LG) inactivated papain *via* conjugate addition of a cysteine group to form (**15**) or, by rearrangement, (**16**), either of which contained a second Michael acceptor capable of reacting with a second active nucleophile and thus leading to irreversible inactivation (Scheme **2**) [29].

Based in this context, Moreira's group reported the synthesis of dipeptide vinyl sultams containing an exocyclic double bond (**17**) (Fig. **11**).



Scheme 2. Mechanism of inhibition of cyclic vinyl sulfones.

Against expectation the dipeptide vinyl sultams were significantly less active ($\approx 10^{-4}$ –fold) than their acyclic analogues, vinyl sulfones. No inhibition could be detected against papain. However, when evaluated against falcipain-2, they showed some activity, albeit modest [30].

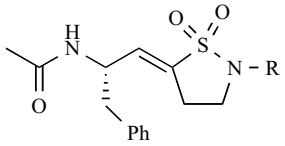


Fig. (11). Vinyl sultams containing an exocyclic double bond (**17**).

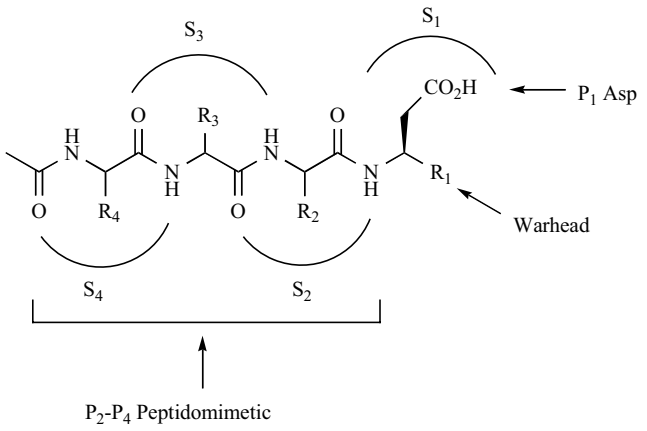
INHIBITORS FOR CLAN CD

There are relatively few classes of Michael acceptors as inhibitors of the clan CD cysteine proteases. And most of them were developed for the enzyme family of caspases. Some caspases are important mediators of inflammation, whereas others are involved in apoptosis [31]. Excessive neuronal apoptosis leads to a variety of diseases such as stroke, Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotropic lateral sclerosis, multiple sclerosis, and spinal muscular atrophy [32]. Thus, potent and specific inhibitors of clan CD proteases could lead to the development of potential new drugs.

Caspases represent one of the most specific protease families yet described, since they have an almost absolute requirement for an aspartic acid residue in the P_1 position of their substrate, and require at least three additional amino acids located in the P_2-P_4 positions [33].

A typical caspase inhibitor can be divided into three structural components: the warhead, the P_1 aspartic acid and the P_2-P_4 peptidomimetic (Scheme **3**) [34].

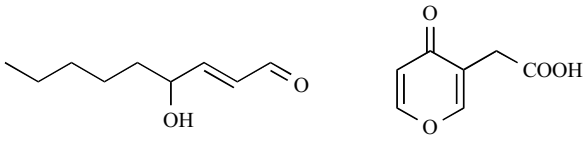
Reversible inhibitors such as peptide aldehydes and irreversible inhibitors such as fluoro- or chloromethyl ketones have been described as substrate based caspase inhibitors [35, 36].



Scheme 3. Schematic of a typical caspase inhibitor.

A few of these inhibitors have been entered preclinical studies with animal models of human diseases. However, the major disadvantage of the inhibitors is their lack of selectivity.

One of the first Michael acceptors described as a caspase inhibitor, was 4-Hydroxynonenal (**18**) (Fig. **12**), a metabolite generated by lipid peroxidation that inhibits recombinant human caspase-1 in a dose-dependent manner [37]. Xylaric acid (**19**), which contains an analogous α,β-unsaturated system, was also described as a caspase-1 inhibitor (Fig. **12**) [38].



18 **19**

Fig. (12). 4-Hydroxynonenal (**18**) and Xylaric acid (**19**).

Graczyk obtained IC_{50} values for recombinant caspase-3 with several other quinones (**20-30**) (Fig. **13**), which suggested that the inhibitory activity correlates with the Michael acceptor abilities of the quinones. They showed no activity against papain [39].

Based on the significant inhibitory activity of simple quinones against caspase-3, his group expected that adding the appropriate peptide chain to a naphtoquinone, would increase the potency and/or selectivity of the inhibitor. The naphtoquinone was chosen as a replacement of the VD fragment in the DEVD sequence (Asp-Glu-Val-Asp) recognized by caspase-3 during PARP cleavage. As expected, the presence of the peptide chain in (**29**) increased inhibitory activity of naphtoquinone moiety against recombinant CPP32 (also known as caspase-3) from IC_{50} = 130 nM to 40 nM.

However, because the absence of the P_1 NH, one could expect that (**29**) may not be able to take advantage of the interaction with Arg341 and Ser339 at the active site of caspase-3 (Fig. **14**).

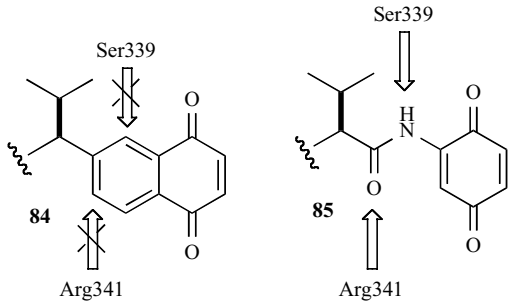


Fig. (14). Interactions at the binding site of caspase-3 with naphtoquinone (**29**) and benzoquinone (**30**).

These interactions could eventually be possible in a benzoquinone scaffold. To determine the potency and selectivity of the benzoquinones (**30a**) and (**30b**), they were assayed against caspase-3 and cathepsin C. As expected the inhibitory activity of (**30a**) and (**30b**) in a caspase-3 assay was higher than that of benzoquinone itself. In contrast, cathepsin C was inhibited weakly with IC_{50} of the order of 1 μM [39].

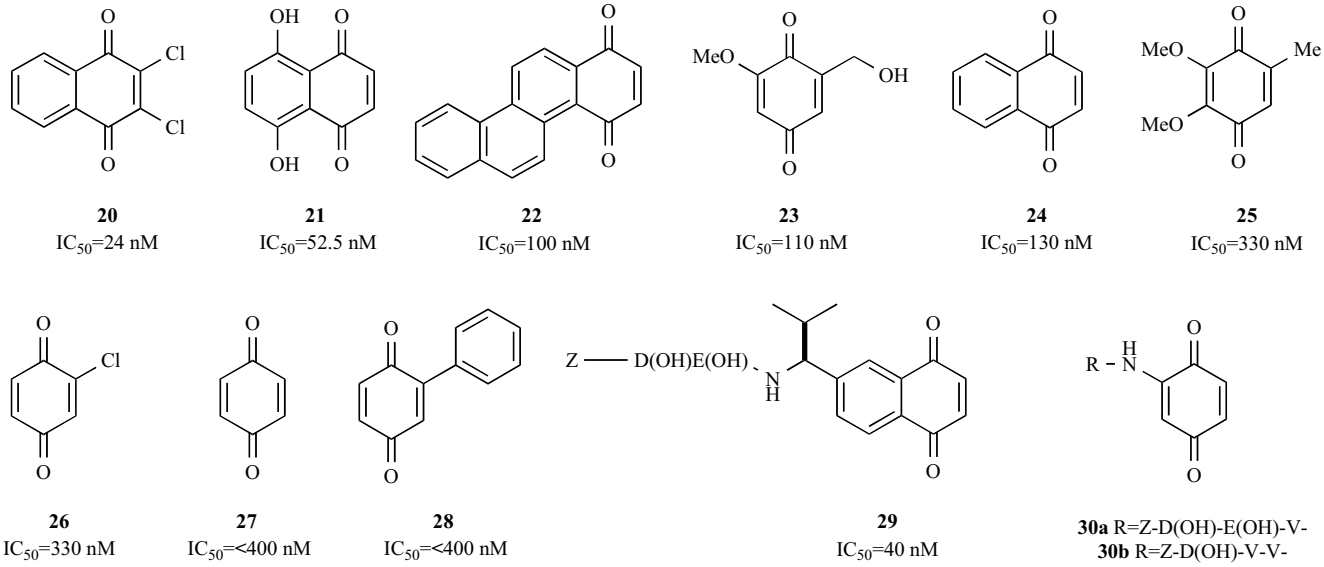


Fig. (13). IC_{50} values for recombinant caspase-3 with selected quinones.

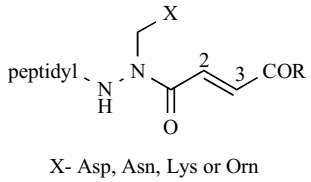
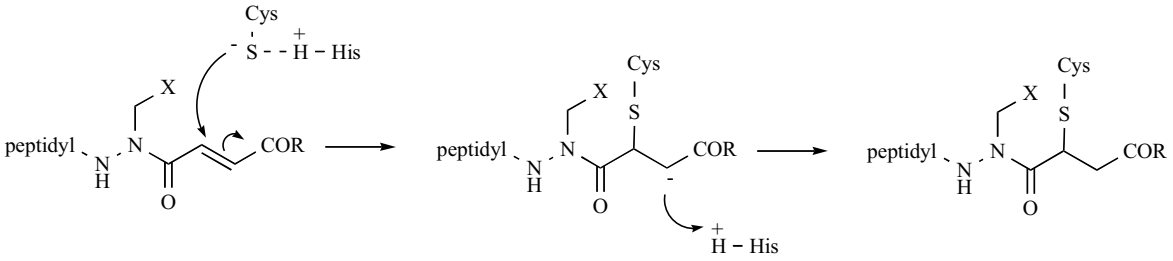


Fig. (15). Aza-peptide Michael acceptor (**31**).

The replacement of the P_1 amino acid α-carbon with a nitrogen results in the formation of the aza-peptide. Aza-peptide Michael acceptors (**31**) with the appropriate P_1 amino acids (Fig. **15**) have been recently described as highly potent and specific for clan CD cysteine proteases. The second-order inhibition rate constants are as high as 6×10^7 M^{-1} s^{-1}. The inhibitors with the appropriate peptide sequence for the targeted enzyme do not show any cross reactivity with clan CA cysteine proteases such as papain, cathepsin B and calpain. There is also little to no cross reactivity toward the other members of clan CD cysteine proteases [40, 41].

The mechanism of inhibition of clan CD proteases by aza-peptide Michael acceptors involve irreversible thioalkylation of the active site Cys by the Michael acceptor by nucleophilic attack at the C-2 carbon on the double bond moiety, followed by protonation of the C-3 carbon to form the thioether derivative (Scheme **4**).



Scheme 4. Mechanism of inhibition of cysteine proteases by aza-peptide Michael acceptors.

From the aza-peptide Michael acceptors synthesized by Powers and co-workers, the inhibitor (**32**) (Fig. **16**) was the most potent and was more selective for caspase-3 over other caspases [41].

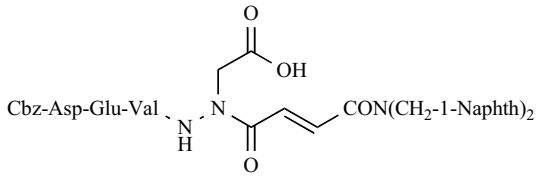


Fig. (16). Aza-peptide Michael acceptor (**32**).

INHIBITORS FOR CLAN PA(C)

Human rhinovirus 3C proteases (3CP) are potently inhibited by Michael acceptors when incorporated into a peptidic recognition element. An extensive SAR study of peptide derived Michael acceptors (**33**) (Fig. **17**) was prompted by Agouron Pharmaceuticals.

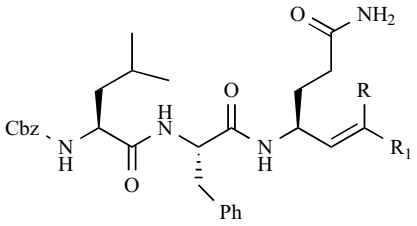


Fig. (17). Structure of the Inhibitors (**33**) developed by Agouron Pharmaceuticals.

These molecules irreversibly inhibited 3CP's from several HRV serotypes and exhibited antiviral activity when tested against these serotypes in cell culture. The first conclusion of the study was that Michael acceptors with trans geometry are better inhibitors of 3CP. Inclusion of an ethyl ester in the inhibitor design (**33a**) (R=H, R_1=CO_2Et) produced both a better enzyme inhibitor and a more potent antiviral agent, while incorporation of an α-fluoro substituent (**33b**) (R=F, R_1=CO_2Et) drastically reduced 3CP inhibitory activity. The related α,β-unsaturated carboxylic acid (**33c**) (R=H, R_1=CO_2H) was a poor 3CP inhibitor and did not exhibit antiviral activity at the highest concentration examined (100 μM).

Amide-containing Michael acceptors displayed reduced anti-3CP activity, poorer antiviral activity, and/or increased toxicity compared to the esters. One exception was a molecule incorporating an indoline derived Michael acceptor [42].

Substituted indolines did not present substantially improved activity when compared with the unsubstituted parent compound. Amides derived from *N*,*O*-dialkylhydroxylamines were also potent 3CP inhibitors. Most of the aliphatic and aryl *α*,*β*-unsaturated ketones displayed increased anti-3CP activity relative to the ester-containing inhibitors. However the ketone–derived Michael acceptors might react rapidly with biological thiols. Vinyl sulfones and molecules which incorporated a vinyl nitrile, vinyl phosphonate, vinyl oxime, and several vinyl heterocycles displayed low levels of anti-3CP inhibition and were poor antiviral agents. In contrast, an compound containing an *α*,*β*-unsaturated nitro moiety, and compounds incorporating acyl lactam, acyl oxazolidinone, and acyl urea funcionalities demonstrated potent, irreversible 3CP inhibitory activity. However, these molecules were inactivated by exposure to nonenzymatic thiols [42].

A molecule containing an *N*-acyl lactam derived Michael acceptor was a potent, irreversible 3CP inhibitor and exhibited good antiviral properties. However, the related carbamate and *N*-methoxy derivatives displayed reduced anti-3CP activity and were poor antiviral agents [42].

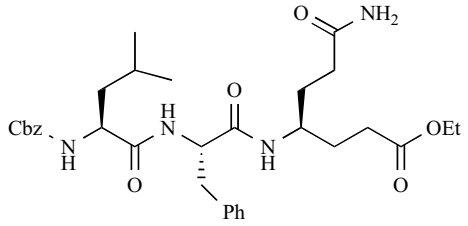
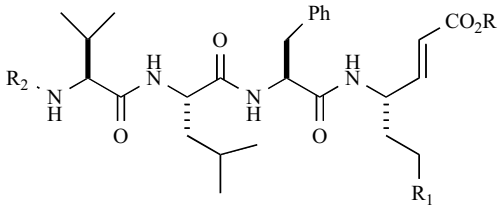


Fig. (18). Structure of inhibitor (**34**).

As expected, compound (**34**) was a relatively weak, reversible 3CP inhibitor and did not display antiviral properties when tested to its solubility limit, which demonstrates the importance of the Michael acceptor moiety in the design of potent 3CP inhibitors [42].

By the same time, Hanzlik also described peptidyl Michael acceptors comprising an appropriate recognition peptide and a vinylogous amino acid ester that inactivate 3CP and inhibit virus replication. The best results were obtained for compounds containing the peptide Boc-V-L-F sequence (Fig. **19**). They gave complete inhibition even at the lowest concentration tested, while others gave only 18-40% inhibition at the highest concentration tested. The IC_{50} values of the more active compounds were all very similar. The inhibition of HRV14-$3C^{pro}$ by Michael acceptors is irreversible and thus covalent as well as stoichiometric. All of the potent compounds are tetra- or pentapeptides having a glutamine-like side chain at P_1 and a hydrophobic side chain at P_4. As Michael acceptors, acrylate esters are much more reactive than the corresponding acrylic acids which are negatively charged at physiological pH values. This may explain why compound (**35e**) was much less effective as an inactivator than its ester analogues (**35a**) and (**35b**). At the P_5 position the enzyme accepts the lipophilic Boc or Cbz groups in (**35a-c**), the blocked glutamate derivative Boc-E(*t*Bu) in (**35d**), or the unblocked zwitterionic glutamyl residue in (**35f**). However, replacement of the P_1 glutamine side chain by a methionine sulfone side chain in (**35g**) leads to substantial loss of activity [43].



35a R = Me; $R_1 = CONH_2$; R_2 = Boc
35b R = Et; $R_1 = CONH_2$; R_2 = Boc
35c R = Me; $R_1 = CONH_2$; R_2 = Cbz
35d R = Me; $R_1 = CONH_2$; R_2 = BocE(tBu)
35e R = H; $R_1 = CONH_2$; R_2 = Boc
35f R = Me; $R_1 = CONH_2$; R_2 = E
35g R = Me; $R_1 = SO_2CH_3$; R_2 = Boc

Fig. (19). Inhibitors of 3CP.

A study of the effect of modification of the peptidyl binding determinant made by Agouron Pharmaceuticals indicated that peptidyl inhibitors composed of at least three amino acids were required for effective recognition of the target protease. Then, a systematic approach was utilized in which one amino acid component of (**33a**) was modified while the other two remained unchanged [44-45]. Based on these results inhibitor (**36**) (Fig. **20**) was conceived, which displayed very rapid, irreversible inhibition of HRV-14 3CP and exhibited potent antiviral activity when tested against HRV-14 in cell culture [44].

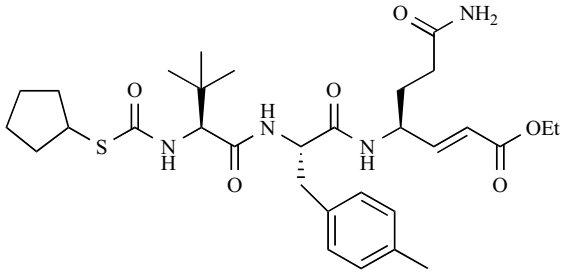


Fig. (20). Structure of inhibitor (**36**).

A series of nonpeptide inhibitors of 3CP was also described by Agouron Pharmaceuticals, as nonpeptide motifs would be expected to have more favorable pharmacokinetic properties. The first study involved benzamide-containing inhibitors (Fig. **21**) [46].

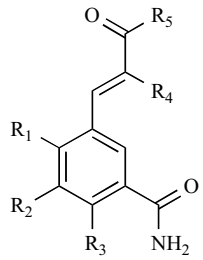


Fig. (21). Structure of the nonpeptide benzamide 3CP inhibitors.

Compound (**37**) (R_1=R_2=R_3=R_4=H, R_5=OEt) was found to be an irreversible inhibitor with a weak inactivation constant of 52 $M^{-1}s^{-1}$. The ester group, in general, was found to have an unremarkable effect on the potency. Increased activation of the Michael acceptor toward nucleophilic addition, by α-cyano substitution (R_4=CN), led to a modest but reversible inhibitor [46].

Unsaturated ketones (R_1=R_2=R_3=R_4=H, R_5=aromatic substituent) displayed potent reversible inhibition but were inactive in the cellular antiviral assay and were found to react with nucleophilic thiols such as DTT [46].

A hydroxymethyl group in R_2 was found to retain all of the potency of the unsubstituted parent (**37**). A parallel synthesis on solid phase was done for the preparation of a large number of benzamide derivatives substituted in the 5-position to access the S_3-S_4 subsites of the enzyme, yet, only modest improvement in enzyme inactivation was realized [46].

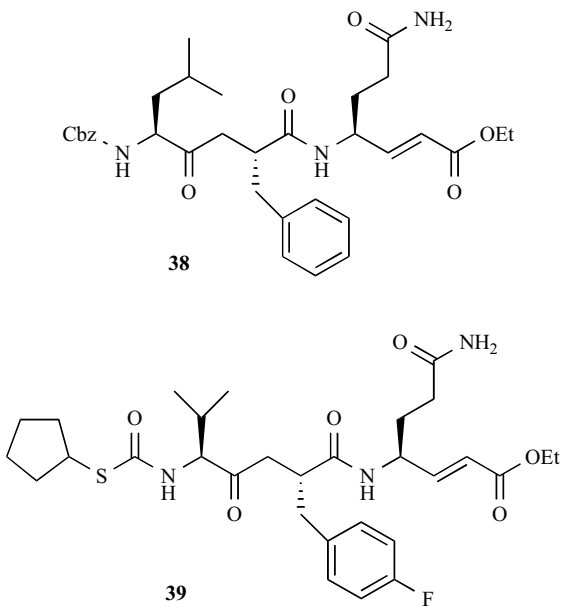


Fig. (22). Ketomethylene-containing inhibitors of rhinovirus 3C.

Ketomethylene-containing inhibitors (Fig. **22**) typically displayed slightly reduced 3CP inhibition activity relative to the corresponding peptide-derived molecules, but they also exhibit significantly improved antiviral properties. The improved antiviral activity was consistent with increased cell membrane permeability due to the reduced potencial of (**38**) to form hydrogen bonds with water. Optimization of the ketomethylene-containing compounds afforded several highly active 3CP inhibitors which displayed potent antiviral activity (EC_{90}=<1μM) against multiple rhinovirus serotypes in cell culture. Compound (**39**) was the most potent inhibitor [47].

P_1-lactam-containing inhibitors display significantly increased 3CP inhibition activity along with improved antirhinoviral properties relative to corresponding l-glutamine-derived molecules. In addition, several lactam-containing compounds exhibit excellent selectivity for HRV 3CP over several other serine and cysteine proteases and are not appreciably degraded by a variety of biological agents [48]. The P_1 lactam moiety was incorporated into tripeptidyl molecules containing a P_3 Val amino acid residue and an N-terminal amide derived from 5-methylisoxazole-3-carboxylic acid [45]. The resulting compounds rapidly and irreversibly inhibited HRV-14 3CP and displayed very potent antiviral properties in cell culture. Inclusion of a Val-Phe ketomethylene dipeptide isostere in the inhibitor design resulted in slightly diminished anti-3CP activity but further improved antirhinoviral properties in cell culture [47]. A tripeptide-derived inhibitor which contained a P_3 tBuGly residue also exhibited impressive anti-3CP and antiviral properties [48].

The above studies led to the selection of one inhibitor (Fig. **23**), Ruprintrivir (AG7088), for clinical development as a nasally delivered antirhinoviral agent. The compound has excellent activity against serotype 14 3CP (K_{obs}/[I] = $1{,}47\times10^6$ $M^{-1}s^{-1}$) and is a potent antiviral agent with low toxicity in the HeLa cell assay (EC_{50} = 0.013 μM; toxic concentration, 50% > 100 μM). In H1-HeLa and MRC-5 cell protection assays, AG7088 inhibited the replication of all HRV serotypes (48 of 48) tested with a mean EC_{50} of 0.023 μM and a mean EC_{90} of 0.082 μM [48-53]. Ruprintivir has advanced to Phase II/III clinical trials.

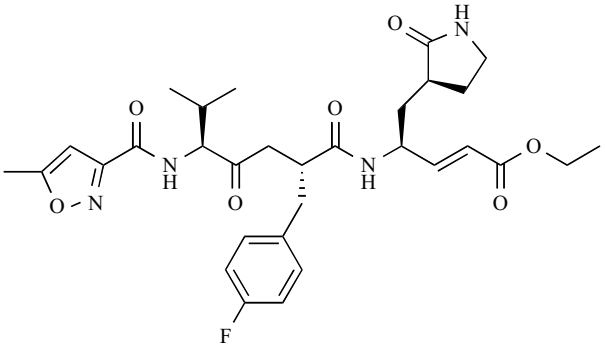
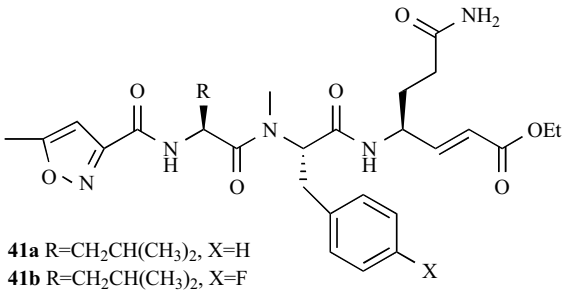


Fig. (23). Ruprintivir (**40**).

Tripeptide-derived molecules incorporating *N*-methyl amino acid residues and C-terminal Michael acceptor moieties (Fig. **24**) were evaluated as irreversible inhibitors of the cysteine-containing human rhinovirus 3CP [54]. Incorporation of an *N*-terminal amide derived from 5-methylisoxazole-3-carboxylic acid (**41a**) improved both 3CP inhibition activity and antiviral properties [45]. In contrast with that observed for non-methylated tripeptidyl 3CP inhibitors, the incorporation of a 4-fluorine (**41b**) resulted in somewhat diminished anti-3CP activity [44]. However, (4-F, *N*-Me) Phe-containing molecules exhibited improved *in vitro* stability relative to the corresponding non-fluorinated inhibitors. Combination of P_3 amino acids containing aromatic side chains (**41c-e**) also afforded several active 3CP inhibitors and antiviral agents [54].



41a R=$CH_2CH(CH_3)_2$, X=H
41b R=$CH_2CH(CH_3)_2$, X=F
41c R=CH_2(1-Naphthyl), X=F
41d R=CH_2(2-Naphthyl), X=F
41e R=CH_2(4-Imidazole), X=F

Fig. (24). Inhibitors of rhinovirus 3C containing *N*-methyl amino acids.

Tripeptidyl C-terminal Michael acceptors with an ester replacement of the P_2-P_3 amide bond (Fig. **25**) were investigated as irreversible inhibitors of the human rhinovirus 3CP by Webber and co-workers. Compared to their amide and ketomethylene counterparts, depsipeptides (**42**) and (**43**) displayed reduced HRV-14 3CP activity, but displayed improved or comparable antiviral activity. Depsipeptide inhibitors may not be ideal therapeutic candidates due to their lack of *in vitro* stability [55].

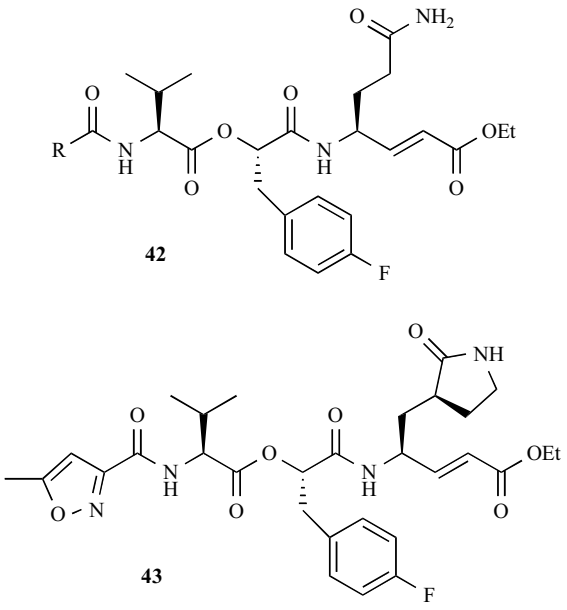


Fig. (25). Depsipeptides (**42**) and (**43**).

Peptidomimetic, 2-pyridone-containing irreversible inhibitors (Fig. **26**) of the HRV 3CP were found to function as potent, broad-spectrum, orally bioavailable antirhinoviral agents. One particular 2-pyridone-containing compound (**44**) was shown to be orally bioavailable in the dog [50]. In stark contrast, the inhibitor was poorly bioavailable when orally delivered in solution to CM-monkeys, probably due to rapid metabolism of (**44**). Reduction of the lipophilicties of the 2-pyridone-containing 3CP inhibitors did not always result in good *in vivo* pharmacokinetics. Nevertheless, two inhibitors (R_2=$CH_2C{\equiv}CH$ and R_2=CH_2CH_3) that exhibited good oral bioavailability in both dogs and monkeys were identified after alteration of the lipophilic P_2 benzyl substituent [56].

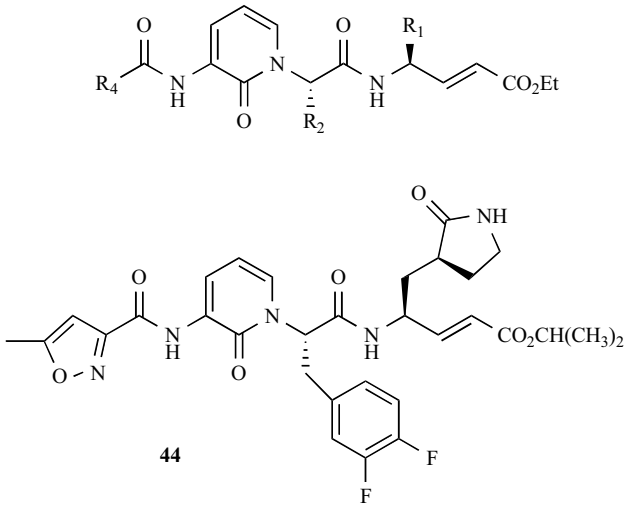


Fig. (26). Structure of 2-pyridone-containing inhibitors of the HRV 3CP.

Incorporation of a bicyclic 2-pyridone moiety resulted in a potent, broad-spectrum antirhinoviral agent (Fig. **27**) [57].

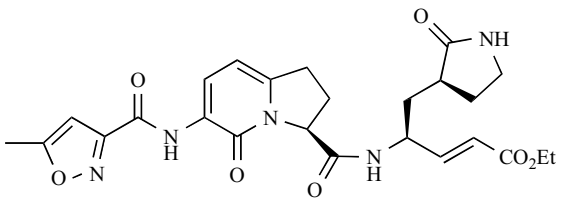


Fig. (27). Bicyclic 2-pyridone-containing inhibitor (**45**).

Utilizing the tools of parallel synthesis and structure-based design, a new class of Michael acceptor-containing, irreversible inhibitors of human rhinovirus 3CP was discovered. Replace of the P_2-P_4 portion of (**33a**) with a smaller, nonpeptidic substituent led to inhibitors that exhibit antiviral activity when tested against HRV-14 infected H1-HeLa cells. All of the compounds contained planar, hydrophobic nature of S_2. The halogenation pattern of the P_2 substituent is shown to have a large influence on activity (Fig. **28**). A properly placed halogen can increase the rate of enzyme inactivation by as much as 30-fold against HRV-14 3CP.

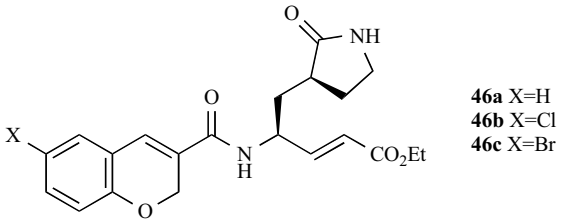


Fig. (28). Nonpeptidic inhibitors of the HRV 3CP.

Despite reduced enzyme inhibition against serotypes other than type 14, these new small molecule inhibitors still maintain slightly better antiviral activity than the larger peptidic (**33a**) [58].

Azodicarboxamide derivatives are also potent irreversible inhibitors of HRV 3C proteinase with IC_{50}'s in the low micromolecular range. Compound (**47**) showed IC_{50} value of 12 μM against HRV 3C proteinase (Fig. **29**). These compounds probably act by adding the active site thiolate to the azo moiety in a Michael fashion to give a covalent complex [59, 60].

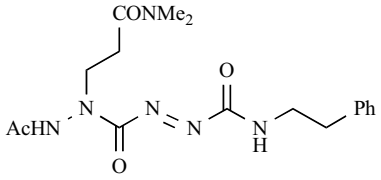


Fig. (29). Azodicarboxamide Michael acceptor inhibitor (**47**).

CONCLUSIONS

Cysteine protease inhibitors have considerable potential utility for therapeutic intervention in a variety of disease states. One type of cysteine proteases inhibitors that has received special attention in the last few years are those based on Michael acceptor scaffolds. Vinyl sulfones and *α,β*-unsaturated carbonyl derivatives have been developed as highly

potent inhibitors for many cysteine proteases of the clans CA and PA(C). Relatively few classes of Michael acceptors inhibitors have been applied to clan CD cysteine proteases.

It has been shown that the peptide sequence and the nature of the substituent on the prime side of the Michael acceptor functionality play an important role in the potency of the inhibitor towards the target enzyme. Thus, the challenge associated with developing irreversible cysteine protease inhibitors will be the design of molecules possessing high selectivity for their target enzymes. Two Michael acceptors inhibitors, K-777 (CRA-3316) and Ruprintrivir (AG7088), developed to treat Chaga's disease and as an antirhinoviral agent, respectively, are already in clinical trials.

ABBREVIATIONS

CM-monkey = Cynomolgus monkey

CPP32 = Cysteine protease P32 (caspase-3)

DPPI = Dipeptidyl peptidase

DTT = Dithiothreitol

E64 = L-*trans*-epoxysuccinyl-leucyl-amido(4-guanidino)butane

Hph = Homophenylalanine

HRV = Human rhinovirus

$MetO_2$ = Methionine dioxide

Mu- = 4-morpholinecarbonyl

PARP = Poly(ADP-ribose) polymerase

SAR = Structure-activity relationships

Z- = Benzyloxycarbonyl.

ACKNOWLEDGEMENTS

This work was supported by the Fundação para a Ciência e Tecnologia (Lisbon, Portugal) by the award of post-doctoral fellowship to M.M.M.S.

REFERENCES

[1] Barrett, A. J.; Rawlings, N. D. *Biol. Chem.*, **2001**, *382*, 727.
[2] Leung-Toung, R.; Zhao, Y. Q.; Li, W. R.; Tam, T. F.; Karimian, K.; Spino, M. *Curr. Med. Chem.*, **2006**, *13*, 547.
[3] Brinen, L. S.; Hansell, E.; Cheng, J. M.; Roush, W. R.; McKerrow, J. H.; Fletterick, R. J. *Structure*, **2000**, *8*, 831.
[4] Rosenthal, P. J.; Sijwali, P. S.; Singh, A.; Shenai, B. R. *Curr. Pharm. Des.*, **2002**, *8*, 1659.
[5] Welburn, S. C.; Coleman, P. G.; Maudlin, I.; Fevre, E. M.; Odiit, M.; Eisler, M. C. *Trends Parasitol.*, **2006**, *22*, 123.
[6] Vicik, R.; Busemann, M.; Baumann, K.; Schirmeister, T. *Curr. Top. Med. Chem.*, **2006**, *6*, 331.
[7] Hernandez, A. A.; Roush, W. R. *Curr. Opin. Chem. Biol.*, **2002**, *6*, 459.
[8] Leung-Toung, R.; Li, W. R.; Tam, T. F.; Karimian, K. *Curr. Med. Chem.*, **2002**, *9*, 979.
[9] Powers, J. C.; Asgian, J. L.; Ekici, O. D.; James, K. E. *Chem. Rev.*, **2002**, *102*, 4639.
[10] O'Brien, T.; Lee, D. *Mini Rev. Med. Chem.*, **2004**, *4*, 153.
[11] Barrett, A. J.; Kembhavi, A. A.; Brown, M. A.; Kirschke, H.; Knight, C. G.; Tamai, M.; Hanada, K. *Biochem. J.*, **1982**, *201*, 189.
[12] Hanzlik, R. P.; Thompson, S. A. *J. Med. Chem.*, **1984**, *27*, 711.
[13] Thompson, S. A.; Andrews, P. R.; Hanzlik, R. P. *J. Med. Chem.*, **1986**, *29*, 104.
[14] Liu, S.; Hanzlik, R. P. *J. Med. Chem.*, **1992**, *35*, 1067.
[15] Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Bromme, D. *J. Med. Chem.*, **1995**, *38*, 3193.
[16] Bromme, D.; Klaus, J. L.; Okamoto, K.; Rasnick, D.; Palmer, J. T. *Biochem. J.*, **1996**, *315*, 85.
[17] Rosenthal, P. J.; Olson, J. E.; Lee, G. K.; Palmer, J. T.; Klaus, J. L.; Rasnick, D. *Antimicrob. Agents Chemother.*, **1996**, *40*, 1600.
[18] Olson, J. E.; Lee, G. K.; Semenov, A.; Rosenthal, P. J. *Bioorg. Med. Chem.*, **1999**, *7*, 633.
[19] Jacobsen, W.; Christians, U.; Benet, L. Z. *Drug Metab. Dispos.*, **2000**, *28*, 1343.
[20] Engel, J. C.; Doyle, P. S.; Hsieh, I.; McKerrow, J. H. *J. Exp. Med.*, **1998**, *188*, 725.
[21] Barr, S. C.; Warner, K. L.; Kornreic, B. G.; Piscitelli, J.; Wolfe, A.; Benet, L.; McKerrow, J. H. *Antimicrob. Agents Chemother.*, **2005**, *49*, 5160.
[22] Sajid, M.; McKerrow, J. H. *Mol. Biochem. Parasitol.*, **2002**, *120*, 1.
[23] Roush, W. R.; Gwaltney, S. L.; Cheng, J. M.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E. *J. Am. Chem. Soc.*, **1998**, *120*, 10994.
[24] Roush, W. R.; Cheng, J. M.; Knapp-Reed, B.; Alvarez-Hernandez, A.; McKerrow, J. H.; Hansell, E.; Engel, J. C. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 2759.
[25] Shenai, B. R.; Lee, B. J.; Alvarez-Hernandez, A.; Chong, P. Y.; Emal, C. D.; Neitz, R. J.; Roush, W. R.; Rosenthal, P. J. *Antimicrob. Agents Chemother.*, **2003**, *47*, 154.
[26] Scheidt, K. A.; Roush, W. R.; McKerrow, J. H.; Selzer, P. M.; Hansell, E.; Rosenthal, P. J. *Bioorg. Med. Chem.*, **1998**, *6*, 2477.
[27] Korver, G. E.; Kam, C. M.; Powers, J. C.; Hudig, D. *Int. Immunopharmacol.*, **2001**, *1*, 21.
[28] Zhu, S.; Hudson, T. H.; Kyle, D. E.; Lin, A. J. *J. Med. Chem.*, **2002**, *45*, 3491.
[29] Iley, J.; Moreira, R.; Martins, L.; Guedes, R. C.; Soares, C. M. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 2738.
[30] Valente, C.; Guedes, R. C.; Moreira, R.; Iley, J.; Gut, J.; Rosenthal, P. J. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 4115.
[31] Denault, J. B.; Salvesen, G. S. *Chem. Rev.*, **2002**, *102*, 4489.
[32] Fleischer, A.; Ghadiri, A.; Dessauge, F.; Duhamel, M.; Rebollo, M. P.; Alvarez-Franco, F.; Rebollo, A. *Mol. Immunol.*, **2006**, *43*, 1065.
[33] Fuentes-Prior, P.; Salvesen, G. S. *Biochem. J.*, **2004**, *384*, 201.
[34] Fischer, U.; Schulze-Osthoff, K. *Cell Death Differ.*, **2005**, *12*, 942.
[35] Callus, B. A.; Vaux, D. L. *Cell Death Differ.*, **2007**, *14*, 73.
[36] Timmer, J. C.; Salvesen, G. S. *Cell Death Differ.*, **2007**, *14*, 66.
[37] Kruman, I.; BruceKeller, A. J.; Bredesen, D.; Waeg, G.; Mattson, M. P. *J. Neurosci.*, **1997**, *17*, 5089.
[38] Salvatore, M. J.; Hensens, O. D.; Zink, D. L.; Liesch, J.; Dufresne, C.; Ondeyka, J. G.; Jurgens, T. M.; Borris, R. P.; Raghoobar, S.; McCauley, E.; Kong, L.; Gartner, S. E.; Koch, G. E.; Pelaez, F.; Diez, M. T.; Cascales, C.; Martin, I.; Polishook, J. D.; Balick, M. J.; Beck, H. T.; King, S. R.; Hsu, A.; Lingham, R. B. *J. Nat. Prod.*, **1994**, *57*, 755.
[39] Graczyk, P. P. *Restor. Neurol. Neuros.*, **1999**, *14*, 1.
[40] Ekici, O. D.; Gotz, M. G.; James, K. E.; Li, Z. Z.; Rukamp, B. J.; Asgian, J. L.; Caffrey, C. R.; Hansell, E.; Dvorak, J.; McKerrow, J. H.; Potempa, J.; Travis, J.; Mikolajczyk, J.; Salvesen, G. S.; Powers, J. C. *J. Med. Chem.*, **2004**, *47*, 1889.
[41] Ekici, O. D.; Li, Z. Z.; Campbell, A. J.; James, K. E.; Asgian, J. L.; Mikolajczyk, J.; Salvesen, G. S.; Ganesan, R.; Jelakovic, S.; Grutter, M. G.; Powers, J. C. *J. Med. Chem.*, **2006**, *49*, 5728.
[42] Dragovich, P. S.; Webber, S. E.; Babine, R. E.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Reich, S. H.; Prins, T. J.; Marakovits, J. T.; Littlefield, E. S.; Zhou, R.; Tikhe, J.; Ford, C. E.; Wallace, M. B.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Harr, J. E. V.; DeLisle, D. M.; Worland, S. T. *J. Med. Chem.*, **1998**, *41*, 2806.
[43] Kong, J. S.; Venkatraman, S.; Furness, K.; Nimkar, S.; Shepherd, T. A.; Wang, Q. M.; Aube, J.; Hanzlik, R. P. *J. Med. Chem.*, **1998**, *41*, 2579.
[44] Dragovich, P. S.; Webber, S. E.; Babine, R. E.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Reich, S. H.; Marakovits, J. T.; Prins, T. J.; Zhou, R.; Tikhe, J.; Littlefield, E. S.; Bleckman, T. M.; Wallace, M. B.; Little, T. L.; Ford, C. E.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; DeLisle, D. M.; Worland, S. T. *J. Med. Chem.*, **1998**, *41*, 2819.
[45] Dragovich, P. S.; Zhou, R.; Skalitzky, D. J.; Fuhrman, S. A.; Patick, A. K.; Ford, C. E.; Meador, J. W.; Worland, S. T. *Bioorgan. Med. Chem.*, **1999**, *7*, 589.

[46] Reich, S. H.; Johnson, T.; Wallace, M. B.; Kephart, S. E.; Fuhrman, S. A.; Worland, S. T.; Matthews, D. A.; Hendrickson, T. F.; Chan, F.; Meador, J.; Ferre, R. A.; Brown, E. L.; DeLisle, D. M.; Patick, A. K.; Binford, S. L.; Ford, C. E. *J. Med. Chem.*, **2000**, *43*, 1670.

[47] Dragovich, P. S.; Prins, T. J.; Zhou, R.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Ford, C. E.; Meador, J. W.; Ferre, R. A.; Worland, S. T. *J. Med. Chem.*, **1999**, *42*, 1203.

[48] Dragovich, P. S.; Prins, T. J.; Zhou, R.; Webber, S. E.; Marakovits, J. T.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Ford, C. E.; Burke, B. J.; Rejto, P. A.; Hendrickson, T. F.; Tuntland, T.; Brown, E. L.; Meador, J. W.; Ferre, R. A.; Harr, J. E. V.; Kosa, M. B.; Worland, S. T. *J. Med. Chem.*, **1999**, *42*, 1213.

[49] Dragovich, P. S. *Exp. Opin. Ther. Pat.*, **2001**, *11*, 177.

[50] Dragovich, P. S.; Prins, T. J.; Zhou, R.; Brown, E. L.; Maldonado, F. C.; Fuhrman, S. A.; Zalman, L. S.; Tuntland, T.; Lee, C. A.; Patick, A. K.; Matthews, D. A.; Hendrickson, T. F.; Kosa, M. B.; Liu, B.; Batugo, M. R.; Gleeson, J. P. R.; Sakata, S. K.; Chen, L. J.; Guzman, M. C.; Meador, J. W.; Ferre, R. A.; Worland, S. T. *J. Med. Chem.*, **2002**, *45*, 1607.

[51] Matthews, D. A.; Dragovich, P. S.; Webber, S. E.; Fuhrman, S. A.; Patick, A. K.; Zalman, L. S.; Hendrickson, T. F.; Love, R. A.; Prins, T. J.; Zhou, R.; Marakovits, J. T.; Zhou, R.; Tikhe, J.; Ford, C. E.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Brothers, M. A.; DeLisle, D. M.; Worland, S. T. *Proc. Natl. Acad. Sci. U.S.A.*, **1999**, *96*, 11000.

[52] Patick, A. K.; Binford, S. L.; Brothers, M. A.; Jackson, R. L.; Ford, C. E.; Diem, M. D.; Maldonado, F.; Dragovich, P. S.; Zhou, R.; Prins, T. J.; Fuhrman, S. A.; Meador, J. W.; Zalman, L. S.; Matthews, D. A.; Worland, S. T. *Antimicrob. Agents Chemother.*, **1999**, *43*, 2444.

[53] Kaiser, L.; Crump, C. E.; Hayden, F. G. *Antivir. Res.*, **2000**, *47*, 215.

[54] Dragovich, P. S.; Webber, S. E.; Prins, T. J.; Zhou, R.; Marakovits, J. T.; Tikhe, J. G.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Ford, C. E.; Brown, E. L.; Binford, S. L.; Meador, J. W.; Ferre, R. A.; Worland, S. T. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 2189.

[55] Webber, S. E.; Marakovits, J. T.; Dragovich, P. S.; Prins, T. J.; Zhou, R.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Srinivasan, B.; Moran, T.; Ford, C. E.; Brothers, M. A.; Harr, J. E. V.; Meador, J. W.; Ferre, R. A.; Worland, S. T. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 2683.

[56] Dragovich, P. S.; Prins, T. J.; Zhou, R.; Johnson, T. O.; Hua, Y.; Luu, H. T.; Sakata, S. K.; Brown, E. L.; Maldonado, F. C.; Tuntland, T.; Lee, C. A.; Fuhrman, S. A.; Zalman, L. S.; Patick, A. K.; Matthews, D. A.; Wu, E. Y.; Guo, M.; Borer, B. C.; Nayyar, N. K.; Moran, T.; Chen, L. J.; Rejto, P. A.; Rose, P. W.; Guzman, M. C.; Dovalsantos, E. Z.; Lee, S.; McGee, K.; Mohajeri, M.; Liese, A.; Tao, J. H.; Kosa, M. B.; Liu, B.; Batugo, M. R.; Gleeson, J. P. R.; Wu, Z. P.; Liu, J.; Meador, J. W.; Ferre, R. A. *J. Med. Chem.*, **2003**, *46*, 4572.

[57] Dragovich, P. S.; Prins, T. J.; Zhou, R.; Johnson, T. O.; Brown, E. L.; Maldonado, F. C.; Fuhrman, S. A.; Zalman, L. S.; Patick, A. K.; Matthews, D. A.; Hou, X. J.; Meador, J. W.; Ferre, R. A.; Worland, S. T. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 733.

[58] Johnson, T. O.; Hua, Y.; Luu, H. T.; Brown, E. L.; Chan, F.; Chu, S. S.; Dragovich, P. S.; Eastman, B. W.; Ferre, R. A.; Fuhrman, S. A.; Hendrickson, T. F.; Maldonado, F. C.; Matthews, D. A.; Meador, J. W.; Patick, A. K.; Reich, S. H.; Skalitzky, D. J.; Worland, S. T.; Yang, M.; Zalman, L. S. *J. Med. Chem.*, **2002**, *45*, 2016.

[59] Hill, R. D.; Vederas, J. C. *J. Org. Chem.*, **1999**, *64*, 9538.

[60] Lall, M. S.; Jain, R. P.; Vederas, J. C. *Curr. Top. Med. Chem.*, **2004**, *4*, 1239.

Received: 15 March, 2007 **Revised: 14 April, 2007** **Accepted: 16 April, 2007**