Monitor: molecules and profiles

Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and biological relevance of new molecules reported in the literature and on the conference scene; *Profiles* offers commentary on promising lines of research, emerging molecular targets, novel technology, advances in synthetic and separation techniques and legislative issues.

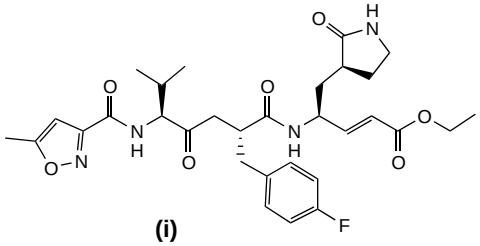
Molecules

Structure-based design of human rhinovirus 3C protease inhibitors

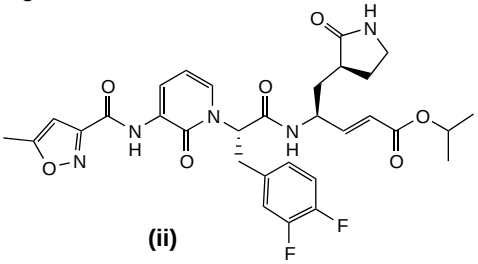
Ruprintrivir (**i**), a substrate based peptidomimetic inhibitor of the human rhinoviral (HRV) 3C protease, is currently in Phase II clinical trials, as a nasally delivered agent for the treatment of the common cold. The compound is an irreversible inhibitor that forms a bond to the active site cysteine residue of the enzyme through a Michael reaction of the α,β-unsaturated ester functionality. The activity of this compound against the enzyme ($k_{obs}/[I]$ = 1,470,000 $M^{-1}s^{-1}$) and HRV (EC_{50} = 0.013 μM) is quite impressive. As described in a recent report, efforts are currently under way to find an analogous compound that can be orally delivered [1].

The X-ray crystal structure of compound **i** bound to HRV 3C protease was used to design a chemically distinct inhibitor to find a bioavailable template. This exercise enabled the replacement of the isopropyl ketone portion of the molecule with a 3-amino-2-pyridone ring, as in compound **ii**. Thorough examination of the remaining portions of the molecule identified the rest of the preferred elements of the inhibitor. This compound is highly active against the enzyme ($k_{obs}/[I]$ = 548,000 $M^{-1}s^{-1}$) and potent against several HRV strains (average EC_{50} = 0.05 μM) but, more importantly, orally bioavailable in the dog (F = 48%, dose = 30 mg kg^{-1}).

1 Dragovich, P.S. *et al.* (2002) Structure-based design, synthesis and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 6. Structure-activity studies of orally bioavailable, 2-pyridone-containing peptidomimetics. *J. Med. Chem.* 45, 1607–1623



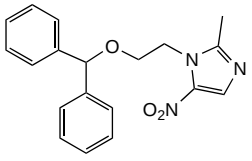
(i)



(ii)

New non-nucleoside reverse transcriptase inhibitors

Molecular modeling and X-ray structural analysis suggest a common motif shared by several non-nucleoside reverse transcriptase inhibitors (NNRTIs), which appears to be a determinant for anti-HIV activity. This structural element is characterized by the arrangement of two π-systems into a 'butterfly-like' orientation. A recent report by Silvestri *et al.* puts this idea to the test by designing and synthesizing an abridged version of this motif contained in a diarylmethane unit [2]. A representative member of this series of inhibitors is compound **iii**.



(iii)

Preliminary SAR studies indicated that anti-HIV-1 activity was highly dependent on the methyl- and nitro-groups attached to the imidazole ring; therefore, the researchers turned their attention towards examining the phenyl groups and the alkyl chain connecting the diarylmethane group to methyl-nitroimidazole. Introduction of substituents such as F, Cl or methyl into the ortho- or meta-position yielded less potent compounds. By contrast, meta-fluoro or meta-methyl analogues showed high anti-viral activity

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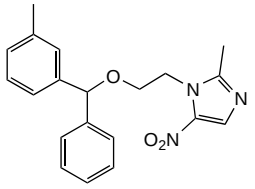
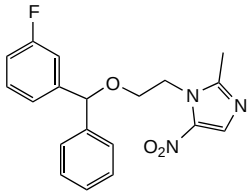
and low cytotoxicity. For example, compounds **iv** and **v** both had EC_{50} values of 0.1 μM against HIV1 in cell culture, cytotoxicity >200 μM and IC_{50} = 0.3 and 0.5 μM (respectively) against reverse transcriptase.



(iv)



(v)

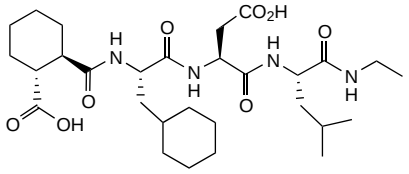
2 Silvestri, R. *et al.* (2002) Synthesis, biological evaluation and binding mode of novel 1-[2-diarylmethoxy)ethyl]-2-methyl-5-nitroimidazoles targeted at the HIV-1 reverse transcriptase *J. Med. Chem.* 45, 1567–1576

'Prime' site binding inhibitors of HCV NS3 protease

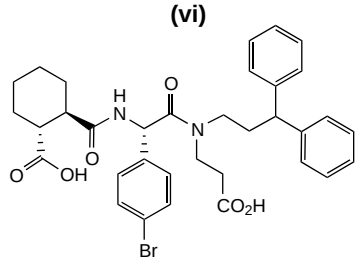
Serine proteases have been targets in several therapeutic areas. One of the more recent applications is inhibition of the hepatitis C virus (HCV) protease. HCV encodes for a serine protease, termed the NS3 protease because of its position as the third non-structural protein contained in the viral genome, which has an essential role in the lifecycle of the virus. Not surprisingly, because HCV is believed to infect up to 170 million people, and there is no generally effective treatment available, pursuit of this target holds much promise.

Peptide-based inhibitors whose sequence is derived from the viral substrates cleaved by the NS3 protease have proven to be highly effective at inhibiting the enzyme. These peptide-based inhibitors acquire most, or all, of their binding energy by filling the non-prime, S_6-S_1 (Schecter and Berger nomenclature) [3], substrate-binding domain of the enzyme. To date, there have been no reports of NS3 protease inhibitors that bind exclusively to the prime, S_1'-S_n', site of the enzyme, which is unfortunate because this domain contains numerous binding pockets. In fact as a whole, there have been few serine protease inhibitors developed so far that make contact with the prime site of the enzyme.

This makes a recent report from Ingallinella *et al*, which describes prime-site binding inhibitors of the HCV NS3 protease of particular interest [4]. Using a noncleavable decapeptide that spanned the P and P′ sites, the researchers were able to identify a tripeptide sequence, which bound to the prime site of the enzyme. This was then used as a template to yield compound **vi**, with a K_i value 13 μM. Investigation of the interaction of this compound with the NS3 protease using circular dichroism, site-directed mutagenesis, a probe displacement assay and NMR, indicated that binding to the prime site was indeed occurring. Further optimization of this lead delivered compound **vii** having reduced peptide character (IC_{50} = 2 μM).



(vi)



(vii)

3 Schecter, I. and Berger, A. (1967) [title] *Biochem. Biophys. Res. Commun.* 27, 157–162

4 Ingallinella, P. *et al.* (2002) Prime site binding inhibitors of a serine protease: NS3/4A of hepatitis C virus *Biochem.* 41, 5483–5492

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Drug Delivery

Improved therapy for yeast infection through use of mucoadhesive thermosensitive gels

Yeast infection is one of the most common gynecological diseases. It is estimated that up to 75% of women are afflicted with at least one yeast infection during their lifetime. The usual treatment for yeast infections, or candidiasis, is antifungal therapy. The most common method of administration is topical treatment because of the systemic toxicity of most antifungal drugs. To be most effective, antifungal agents need to reside at the site of infection for prolonged periods. Conventional over-the-counter gel formulations for vaginal candidiasis do not remain at the site for long, leading to frequent dosing requirements, usually once a day or more for several days to a week. Patient compliance can be low because of the inconvenience of the regimen. Complications include recurrent infection, often in a form that is resistant to the antifungal agent. More aggressive prescription regimens are often required in these instances, leading to more inconvenience for the patient.

Clotrimazole (CT) is a commonly used antifungal for the treatment of vaginal candidiasis. Chang and co-workers have recently reported a mucoadhesive formulation with prolonged antifungal activity based on a combination of poloxamers and polycarbophil to deliver CT [1]. This mucoadhesive thermosensitive gel (MTG) formulation could lend many advantages based on a prolonged drug residence time at the site of infection. Antifungal activity of the MTG formulation of CT was tested *in vivo* against *Candida albicans* vaginitis in female rats. The formulation exhibited significantly prolonged activity over a currently available formulation, as well as better viability of epithelial cells.

MTG formulations were prepared using the cold method. Briefly, polycarbophil (PC) was slowly added to citrate-phosphate

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