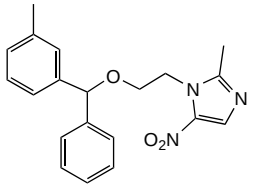
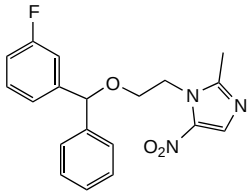
and low cytotoxicity. For example, compounds **iv** and **v** both had EC_{50} values of 0.1 μM against HIV1 in cell culture, cytotoxicity >200 μM and IC_{50} = 0.3 and 0.5 μM (respectively) against reverse transcriptase.



(iv)



(v)

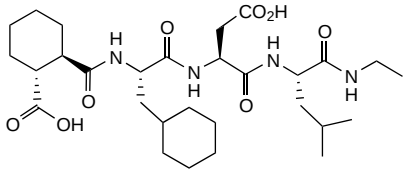
2 Silvestri, R. *et al.* (2002) Synthesis, biological evaluation and binding mode of novel 1-[2-diarylmethoxy)ethyl]-2-methyl-5-nitroimidazoles targeted at the HIV-1 reverse transcriptase *J. Med. Chem.* 45, 1567–1576

'Prime' site binding inhibitors of HCV NS3 protease

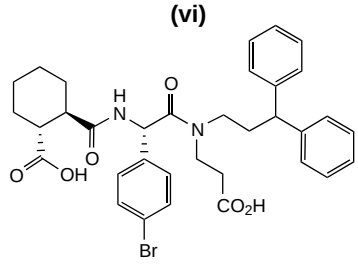
Serine proteases have been targets in several therapeutic areas. One of the more recent applications is inhibition of the hepatitis C virus (HCV) protease. HCV encodes for a serine protease, termed the NS3 protease because of its position as the third non-structural protein contained in the viral genome, 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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buffer (0.1 M, pH 4.0) at 4 °C. An appropriate mixture of poloxamers P407® and P188® (BASF, http://www.basf.com) were added to the PC solution and allowed to dissolve overnight at 4°C. CT was dissolved in a mixture of ethanol and polyethylene glycol (PEG) 400 (3:5) and added to the above solution. Mixtures of varying ratios were tested for mucoadhesive strength, the work required to expel them from a syringe, *in vivo* antifungal activity, and effect on cellular viability.

High mucoadhesive strength in an MTG formulation is an advantage because it increases the residence time of the antifungal agent on the infected vaginal tissues. Simultaneously, the 'syringability' of the formulation – the ease by which the gel can be applied from a syringe or tube – must be considered for convenient vaginal application. A texture analyzer in adhesion mode was used to measure the mucoadhesive strength; the same instrument in compression mode was used to measure the work required to expel the formulations from a syringe. Mucoadhesive strengths of MTG formulations varied with the content of PC and P188. As the composition of P188 increased from 15% to 20%, the mucoadhesive strengths increased. When the composition ratios of P407:P188 remained constant, the mucoadhesiveness increased with increased PC content. However, formulations with higher PC content suffered from lower syringability. A formulation composed of P407:P188:PC (15:20:1.0) exhibited maximum mucoadhesiveness but required the most work to expel the formulation from the syringe. For a balance between the properties of high mucoadhesiveness and higher syringability, two MTG formulations were chosen for further study. Both formulations had a PC content of 0.2%, with two different P407:P188 ratios, P407:P188:PC ratios of 15:15:0.2 (MTG1) or 15:20:0.2 (MTG2).

To test the antifungal activity of CT-containing MTG formulations, vaginal candidiasis was induced in female rats. Because this model is hormone dependent, pseudo-estrous was induced by pretreatment with estradiol benzoate and pseudo-estrous was maintained throughout the study. The rats were challenged with *C. albicans* and at 48 h after challenge a well-established infection resulted. A control group with no drug treatment maintained a similar level of infection throughout the study, indicating that the animals received proper doses of estrogen and challenge inoculum.

MTG formulations were dosed intravaginally to anesthetized rats. At predetermined time points, vaginal lavage samples were collected. The lavage samples were plated, incubated, and colony count values recorded. MTG formulations showed highly prolonged antifungal activity of CT over 10 days. A PEG-based conventional formulation showed a significant reduction on day 4 but did not completely eliminate *C. albicans* infection. The mean colony counts of the PEG-treated group increased from day 7 and showed no significant differences from control on day 10. By contrast, both MTG formulations resulted in continuous reduction in the concentration of *C. albicans* until 10 days post-dose. Of the two formulations, the MTG1 formulation exhibited a higher release rate of CT *in vitro* versus MTG2. MTG1 also resulted in a more rapid drop in the concentration of *C. albicans* at the initial phase of post-treatment. The concentration of *C. albicans* in vaginal tissues were more than 10^4-fold lower in both MTG-treated groups versus untreated control.

Based on the effectiveness of the MTG formulations in treating *C. albicans* infection, the safety of the gels in cervical epithelial cells was tested. Cell viability of cervical epithelial tissue was investigated over different concentrations of CT in phosphate buffer (PBS) and MTG gels. CT reduced the viability of the cells in a dose-dependent manner, but the MTG vehicles alone did not influence the viability of the cells. The use of MTG vehicle significantly reduced the cytotoxicity of CT. The concentrations of CT exhibiting 50% cell viability were 53 and 70 μg ml^{-1} for CT in PBS and MTG, respectively. By contrast, PEG-based formulations highly increased the cytotoxicity of CT. MTG also did not alter the morphology of vaginal tissues. Compared to untreated control, the MTG-treated group showed no visible sign of inflammation or necrosis.

These results indicate that a MTG formulation of CT has advantages in the antifungal treatment of vaginal candidiasis. The mucoadhesive properties increase residence time on the infected vaginal tissues and, in this study, exhibited increased and prolonged antifungal activity versus a conventional PEG-based formulation. In addition, cell viability and morphology support the safety of MTG gels for vaginal application. Syringability was also taken into account for convenience of application by the end user. Further successful development could lead to a more convenient and effective treatment for yeast infection.

1 Chang, J.Y. *et al.* (2002) Prolonged antifungal effects of clotrimazole-containing mucoadhesive thermosensitive gels on vaginitis *J. Control. Release* 82, 39-50

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