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.

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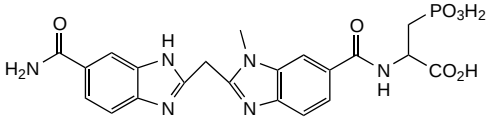
Novel antiviral molecules

Inhibitors of HCV-protease

The NS3 serine protease expressed by the hepatitis C virus (HCV) is one of the most prominent targets currently being explored in an effort to discover therapeutic agents to treat infection. Publications have recently appeared that highlight the progress of three different programs aimed at developing inhibitors of the enzyme.

Sperandio *et al.* report the discovery of non-peptide inhibitors based on a bis-benzimidazole-containing template [1]. Compound **i** (APC6336) was discovered during a screen of a diverse library of bis-benzimidazole analogues. In the presence of Zn^{2+} it is a highly potent, reversible and competitive inhibitor (K_i = 0.2 μM) but it is much less active in the absence of Zn^{2+} (K_i = 170 μM). Previous results with this bis-benzimidazole template indicate that it binds to the catalytic sites of certain serine proteases by a Zn^{2+} ion, which is coordinated to the conserved His57 and Ser195 residues [2]. The effect of Zn^{2+} on the binding affinity of **i** suggests that it binds to the NS3 protease in the same fashion. The negatively charged phosphonate-containing residue also appears to be important for inhibition because the corresponding phosphonate ester was 10-times less active. Moreover, increasing the amount of negative charge on the molecule by the addition of two more phosphoalanine residues improves the binding to NS3.

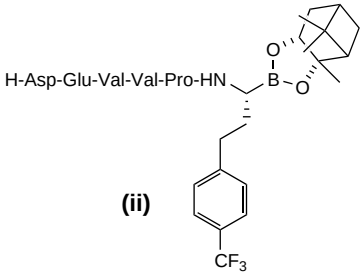


(i)

The second report comes from the labs of Bristol-Myers Squibb (http://www.bms.com) and details the SAR of the P1 position (Schecter and Berger nomenclature [3]) of a peptidomimetic scaffold containing a C-terminal boronic acid group [4]. The corresponding S1 site on the enzyme is not well defined but consists of a broad surface extending to the S3 site. The enzyme usually prefers small hydrophobic groups at the P1 site but the current research shows that phenethyl-based substituents are also accommodated.

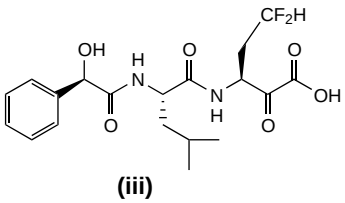
The investigation to uncover the SAR at the P1 position culminated in compound **ii**, which was found to have excellent activity against the NS3 protease (K_i = 0.002 μM). The boronate ester of this compound readily hydrolyzes to the corresponding boronic acid, which is the active form. Optimal activity and selectivity over other serine proteases was achieved by including the para-trifluoromethyl group.

The SAR of the P3 residue of a peptidomimetic template is explored in the third disclosure [5]. The discovery of tripeptide-based



(ii)

inhibitors containing an α-ketoacid moiety at the C-terminus has previously been published [6]. This template features a difluoroethyl side chain, which mimics the side chain of cysteine and a ketone group positioned to covalently bind the active serine residue. In the current investigation, the core dipeptide – consisting of leucine attached to the ketoacid cysteine mimetic – was used as a template to explore non-aminoacid based P3 substituents. Thus, it was found that α-hydroxy carboxylic acids containing a lipophilic side chain, such as that in compound **iii**, could effectively replace the P3 amino acid. Contrary to initial expectations, the preference for the R-configuration at this site suggests that the α-hydroxy group does not interact with the enzyme. The crucial binding element



(iii)

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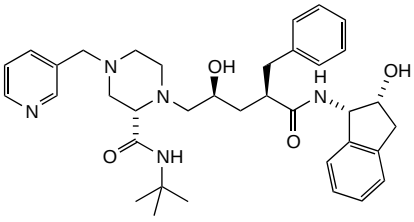
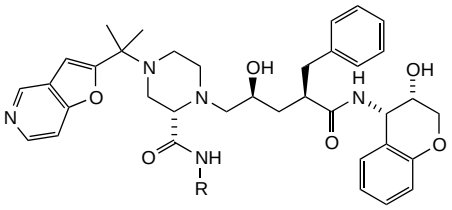


Indinavir



(iv), R = t-butyl
(v), R = -CH_2CF_3

contained within this P3 moiety appears to be the lipophilic side chain.

1 Sperandio, D. *et al.* (2002) Highly potent non-peptidic inhibitors of the HCV NS3/NS4A serine protease *Bioorg. Med. Chem. Lett.* 12, 3129–3133
2 Katz, B. A. *et al.* (1998) Design of potent zinc-mediated serine protease inhibitors *Nature* 391, 608–612
3 Schecter, I. and Berger, A. (1967) On the size of the active site in proteases. I Papain. *Biochem. Biophys. Res. Commun.* 27, 157–162
4 Priestly, E. S. *et al.* (2002) P1 Phenethyl peptide boronic acid inhibitors of HCV NS3 protease *Bioorg. Med. Chem. Lett.* 12, 3199–3202
5 Nizi, E. *et al* (2002) Capped dipeptide α-ketoacid inhibitors of the HCV NS3 protease *Bioorg. Med. Chem. Lett.* 12, 3325–3328
6 Colarusso, S. *et al.* (2002) Evolution, synthesis and SAR of tripeptide α-ketoacid inhibitors of the hepatitis C virus NS3/NS4A serine protease. *Bioorg. Med. Chem. Lett.* 12, 705–708

New analogues of indinavir

In the field of anti-HIV therapeutics, the development of viral drug resistance is a given and the HIV-protease inhibitors are no exception. For those protease inhibitors that are currently approved for the treatment of HIV infection, the development of resistance through viral mutations has been observed in patients [7]. Even so, it is believed that the development of resistance can be suppressed by increasing drug exposure relative to drug potency ratio. This can be accomplished in two ways, either by making key structural changes, which improve pharmacokinetics (PK) – for instance by blocking metabolic sites – or by increasing potency.

Two recent publications from Merck (http://www.merck.com) describe attempts to accomplish both of these goals through new P3 and P2′ (Schecter and Berger nomenclature [8]) analogues of indinavir, which posses either improved PK or improved potency [9,10].

The major sites of metabolism of indinavir have been determined to be in the P3 and P2′ regions of the molecule, namely the nitrogen of the pyridine ring, the methylene linker connecting the pyridyl group to the piperazine ring and the methylene group of the aminoindanol ring. In compound **iv**, all of these sites have been altered in an attempt to block the metabolism. This results in an improvement in exposure in the dog, as indicated by an increase in bioavailability, C_{max}, AUC and $T_{1/2}$. Moreover, compound **iv** is approximately 10-times more active as an inhibitor of HIV-protease *in vitro* and against the virus in cell culture.

Further improvements in potency and PK were achieved when the t-butyl amide moiety of **iv** was changed to the corresponding trifluoroethyl amide found in compound **v**. Moreover, **v** showed a twofold increase in plasma exposure over **iv**, as measured by C_{max} and AUC. However, the improvement in exposure could be the result of an increase in CYP3A4 and CYP2D6 inhibition observed for this compound. Previous work has shown that CYP3A4 is the major P450 isoform responsible for the metabolism of indinavir.

7 Boden, D. and Markowitz, M. (1998) Resistance to human immunodeficiency virus type 1 protease inhibitors. *Antimicrob. Agents Chemother.* 42, 2775–2783
8 Schecter, I. and Berger, A. (1967) On the size of the active site in proteases. I Papain *Biochem. Biophys. Res. Commun.* 27, 157–162
9 Cheng, Y. *et al.* (2002) Indinavir analogues with blocked metabolism sites as HIV protease inhibitors with improved pharmacological profiles and high potency against PI-resistant viral strains *Bioorg. Med. Chem. Lett.* 12, 2419–2422
10 Duffy, J. L. *et al.* (2002) Synthesis and activity of novel HIV protease inhibitors with improved potency against multiple PI-resistant viral strains *Bioorg. Med. Chem. Lett.* 12, 2423–2426

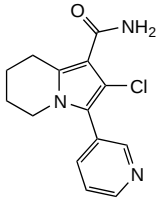
New potential treatment of HCMV infection

Human cytomegalovirus (HCMV) is a member of the herpes virus family and is one of the major pathogens affecting those with compromised immune systems, such as AIDS and transplant patients. Although therapies are available to treat HCMV infection, these drugs – ganciclovir, foscarnet and cidofovir – all act by blocking the viral DNA polymerase and are associated with toxic side effects. In addition, viruses that are resistant to these agents have been observed in the clinic.

Efforts to discover new agents that target other steps of the viral lifecycle and are structurally unique from available drugs are under way. For example, a structurally unique lead compound, CMV423 (**vi**), has recently been described in the literature and targets a different step in the HCMV lifecycle and is active against resistant viral strains [11].

CMV423 was discovered in a drug screening effort and proved to be active in cell culture plaque-reduction assays (IC_{50} = 0.004–0.007 μM) compared with the clinically approved agents; cidofovir, ganciclovir and foscarnet were considerably less active (IC_{50} = 0.12–55 μM) against the same viral strains. Moreover, CMV423 was found to be active against drug resistant HCMV strains isolated in the clinic or selected in cell culture.

Although HCMV is a herpes virus, CMV423 was much less active against other members of this family, such as HSV1 (herpes virus 1), HSV2 and VZV (varicella zoster virus) but it appears to



(vi)

possess some activity against HSV6. The mechanism of action is unknown, but time-of-addition studies and HCMV antigen-expression measurements suggest that it is acting at a stage of the viral life-cycle preceding DNA polymerization. Therefore, compound **v** or a related analogue could represent a new generation of agent to treat HCMV infection.

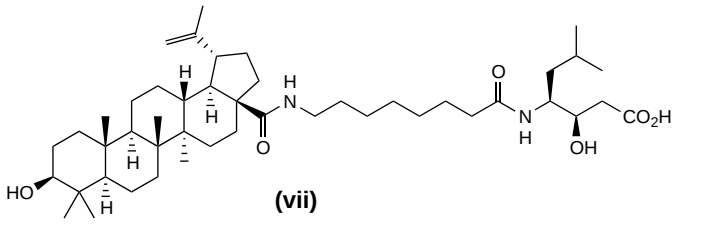
11 Snoeck, R. *et al.* (2002) 2-Chloro-3-pyridin-3-yl-5,6,7,8-tetrahydroindolizine-1-carboxamide (CMV423), a new lead compound for the treatment of human cytomegalovirs infections *Antiviral Research* , 55, 413–424

Betulinic acid analogues as HIV-fusion inhibitors

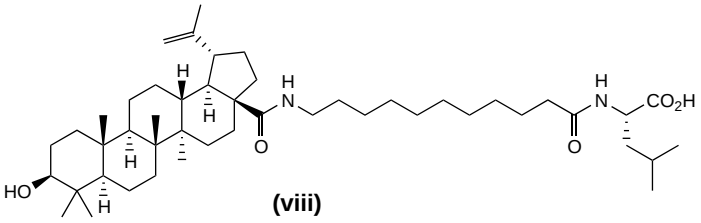
Viral entry is an overly broad term used to encompass the unique steps carried out by HIV, which results in the passage of the viral components into the cytosol of the cell. This process includes attachment of the virus to its receptor and co-receptor, insertion of the viral fusion proteins into the cell membrane, fusion between the cellular and viral membranes, entry of the viral particle into the cytosol, then release of the viral proteins and genetic material. Each of these steps has been targeted as a potential site for the development of new anti-HIV drugs.

Enfuvirtide (T20) – an agent directed at the fusion step – has recently shown promise in treating HIV-infection in the clinic, thus indicating the potential of targeting this step [12]. However, this drug is a 36 amino acid peptide delivered by subcutaneous injection. A small-molecule fusion inhibitor could offer an advantage over T20 in terms of patient compliance, provided the compound could be administered orally.

Betulinic acid analogues, such as IC9564 (**vii**), have been found to inhibit HIV in cell culture (EC_{50} = 0.04 μM) by blocking membrane fusion [13]. Therefore, this chemotype appears to be a suitable lead for the development of a small-molecule fusion inhibitor. An attempt to improve the activity of this series of compounds by modifying the isopropylene group and the C28 side chain has recently been disclosed.



(vii)



(viii)

Saturation of the isopropylene group of compound **vii** had no noticeable effect on anti-HIV activity. Replacing the statin amino acid with isoleucine resulted in a four-times decrease in activity but the potency could be restored by lengthening the methylene side chain as shown for compound **viii** (EC_{50} = 0.05 μM).

These modifications did not improve the overall activity but they do suggest that the isopropylene side chain is not important for activity and that the statin moiety can be replaced by a less complex amino acid, provided that the spacing of the carboxylic acid terminus with respect to the terpene portion of the molecule is adjusted.

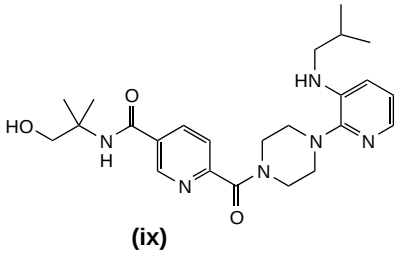
12 Chen, R. Y. *et al.* (2002) Enfuvirtide. *Expert Opinion. Investig. Drugs* 11, 1837–1843

13 Holz-Smith, *et al.* (2001) Role of human immunodeficiency virus (HIV) type 1 envelop in the anti-HIV activity of the betulinic acid derivative IC9564 *Antimicrob. Agents Chemother.* 45, 60–66

Non-nucleoside hepatitis B virus polymerase inhibitors

The hepatitis B virus (HBV) is believed to infect up to 300 million people worldwide. Chronic infection with the virus increases the risk of permanent liver damage, such as cirrhosis and liver cancer. Several nucleoside analogues that inhibit the polymerase expressed by the virus have recently been approved or are currently under development, including lamivudine, adefovir and entecavir. These agents are believed to act as DNA chain terminators after being phosphorylated and attached to the growing viral DNA-chain. As such, these drugs rely on cellular kinases for activation.

Interestingly, a non-nucleoside polymerase inhibitor has been reported from the labs of the DongWha Pharmaceutical Company (http://www.dong-wha.co.kr) [14]. The non-nucleoside class of inhibitors is unique from the nucleoside-based compounds in that phosphorylation is not required for activation and the compounds presumably bind a different site on the enzyme. This implies that the activity of these agents will not be affected by cellular kinase levels and nucleoside resistance mutations. Compound **ix** is one of the more optimal compounds disclosed, having an IC_{50} value of 1 ng ml^{-1} against the enzyme.



(ix)

14 Lee, J-S. *et al.* (2002) 2,5-Pyridinecarboxylic acid derivatives as non-nucleoside reverse transcriptase inhibitors of hepatitis B virus. *Bioorg. Med. Chem. Lett.* 12, 2715–2717

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