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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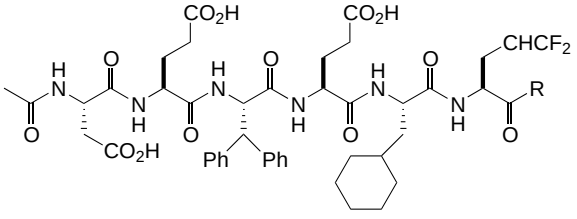
Novel antiviral molecules

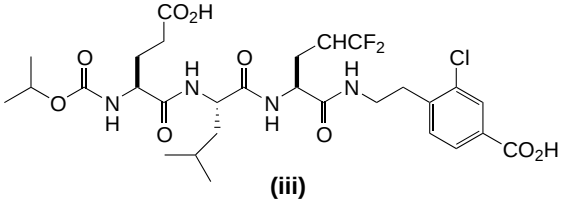
Discovery and initial SAR of novel inhibitors of Hepatitis C protease

The NS3-serine protease that is expressed by the Hepatitis C virus (HCV) has emerged as an established target for the development of potential anti-viral agents to treat infection. Unlike most serine proteases, the Achilles' heel of NS3 is its susceptibility to strong product inhibition. This has led to the discovery of highly potent inhibitors, a recent example of which is BILN-2061, discovered by Boehringer Ingelheim (http://www.boehringer-ingelheim.com) [1]. This compound has demonstrated encouraging results in Phase I and II clinical trials. A different series of inhibitors, based on the hexapeptide lead, compound **i** (IC_{50} = 0.04 μM), was discovered by a group at IRBM (Istituto di Ricerche di Biologia Molecolare P. Angeletti; http://www.irbm.it). Interestingly, a recent report demonstrates that phenethyl amide derivatives of compound **i** are more potent than the parent carboxylic acids [2]. This discovery might offer a pathway towards reducing the overall size of compounds similar to compound **i**.

The available evidence suggests that product-based inhibitors bind to the non-prime (Schecter- Berger nomenclature) substrate domain of the NS3-protein with the C-terminal carboxylic acid occupying the active site domain of the protease. It is likely that the C-terminal carboxylic acid of compound **i** associates with the oxyanion hole of the protease active site, therefore, it was surprising to find that the C-terminal benzylamide (**ii**) (IC_{50} = 0.004 μM) was a more potent inhibitor of NS3 than the parent acid. The increase in activity might be consistent with the binding model mentioned above, as it would place the phenethyl amide group in the non-prime binding domain of the enzyme. Thus, the loss of the interaction with the oxyanion hole is compensated for by establishing a new interaction with the protease non-prime region. Assuming this to be the case, the molecule was pruned back to yield a moderately active, smaller, less charged analogue (**iii**) (K_i = 0.6 μM). The substituents on the benzyl ring where found to increase activity of the tripeptide over the corresponding unsubsituted phenylamide. It is likely that further modifications will be required to improve the activity of the tripeptide phenethylamide (**iii**) to achieve clinical efficacy. Unfortunately, conventional techniques for improving potency often involve the addition of a hydrophobic or charged functional group.



(i), R = -OH
(ii), R = -NH$(CH_2)_2$Ph



(iii)

1 Llinas-Brunet, M. *et al.* (2003) Discovery of BILN 2061: a small-molecule inhibitor of the hepatitis C virus serine protease MEDI-320. *Proc. 225th ACS National meeting*
2 Colarusso, S. *et al.* (2003) Phenethyl amides as novel noncovalent inhibitors of Hepatitis C virus NS3/4A protease: discovery, initial SAR, and molecular modeling. *J. Med. Chem.* 46, 345–348

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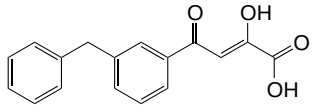
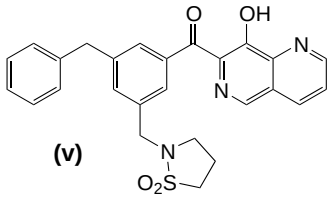
Novel inhibitors of HIV integrase

The viral fusion inhibitor T-20 notwithstanding, current therapy for HIV infection is primarily directed at the viral polymerase and protease. It is generally believed that agents directed at other targets are needed and will be required in the future as HIV has evolved resistance to all known classes of drugs. Although a third viral enzyme, HIV integrase, has long been recognized as a potential target for the development of agents to combat HIV-infection, finding a clinically useful candidate has proven to be difficult. A number of promising leads in this field have evaporated, due to compound reactivity and/or off-target activity, disguised as integrase inhibitory activity. However, a significant breakthrough was reported by Merck (http://www.merck.com), when it was found that certain compounds, such as compound **iv**, possessing a 1,3-diketoacid moiety selectively inhibited the enzyme (IC_{50} = 0.01 μM), resulting in potent antiviral activity in cell culture (CIC_{95} = 1.11 μM) [3]. Being diketoacids, these compounds were not regarded as clinical leads due to concerns about stability in vivo. Nonetheless, these compounds are important because they help to define a pharmacophore for inhibition of integrase.



(iv)

A recent disclosure describes the evolution of the diketoacid into a chemotype that is more suitable as a clinical candidate [4]. Despite the acidic nature of the terminal carboxylate group of compound **iv,** it is believed that one of the oxygen atoms of this group functions as a Lewis base. This has enabled the replacement of the diketoacid portion of the molecule by a hydroxyquinoline-based template, as in compound **v** (IC_{50} = 0.01 μM, CIC_{95} = 0.39 μM). The



(v)

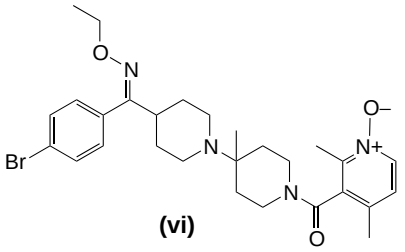
additional nitrogen that is present in this template was installed to avoid steric interaction between the phenyl rings that are attached to the central carbonyl. The sultam group, appended onto the central phenyl ring, imparts additional activity in cell culture, although the reason for this is not yet clear. As expected, in cell culture, virus, containing mutations in integrase that yield resistance to compound **iv,** is also resistant to compound **v,** indicating that both compounds inhibit HIV-1 by targeting integrase at the same site. Naphthyridine (**v**) displays a favorable pharmacokinetic profile in rats dosed at 2 mg kg^{-1}, having low plasma clearance (CL_p = 2.98 ml $min\ kg^{-1}$) and a long $T_{1/2}$ (9.7 h). There is no mention of compound **v** being pursued as a clinical candidate; however, the insights gained in its discovery probably resulted in the discovery of L-870810 a structurally related compound that has entered the clinic (discussed by D. Hazuda at the XI International Workshop on HIV Drug Resistance, July 2–5, Seville, Spain).

3 Hazuda, D. *et al.* (2000) Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* 287, 646–650

4 Zhuang, *et al.* (2003) Design and synthesis of 8-hydroxy-[1,6]naphthyridines as novel inhbitors of HIV-1 integrase *in vitro* and in infected cells. *J. med. Chem.* 46, 453–456

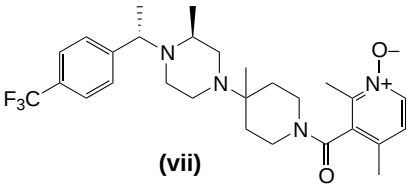
New CCR5 antagonists with potent activity against HIV-1

Since the discovery that HIV-1 binds to CD4 and one of two chemokine receptors CCR5 or CXCR4 to gain entry into the cell, efforts have been undertaken to develop inhibitors directed towards this step in the viral lifecycle. Early studies indicated that the endogenous ligands of the chemokine receptors prevented



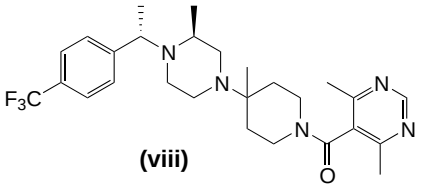
(vi)

infection of HIV-1, presumably by blocking the binding of the virus to the receptor [5]. These results were coupled with the observation that individuals who were homozygous for a mutation in CCR5 were resistant to infection, suggesting that a selective antagonist of the CCR5 receptor could be used to treat infection without incurring mechanism-based side effects [6]. Recently, some promising clinical candidates have emerged, for example SCH-C (**vi**), a first generation CCR5 antagonist and SCH-D (**vii**), a related second-generation compound.



(vii)

A recent disclosure from Schering-Plough (http://www.spcorp.com) provides some understanding of the evolution of the chemotype from the first generation antagonist towards the second-generation compound [7]. An early prototype, Sch-350634 (**viii**), possessing a piperazine-based template similar to that of SCH-D was found to have high affinity for CCR5 (K_i = 7 nM) and potent activity against HIV isolates in cell culture (IC_{50} = 2-20 nM), but it also showed affinity for the muscarinc receptors M1



(viii)

and M2 (K_i = 350 nM and 250 nM, respectively) [8]. Interestingly, the dimethylpyridine amide of compound **vii** existed as a mixture of four slowly equilibrating rotamers, separable by chiral HPLC. Modifications to the pyridine amide were, thus, examined to reduce M1/M2 binding and simplify the chemical analysis of the product. Pyrimidine (**viii**) exists a mixture of two rotamers, due to symmetry, and yields improved activity in cell culture (IC_{50} = 0.5 nM, HIV cell entry). In addition, muscaranic receptor binding appears to be attenuated somewhat (K_i (M2)= 456 nM, K_i (M1) 575 nM) over compound **vii**. This compound also showed good absorption (F = 97% and 50% in rat and dog, respectively), exposure (AUC = 22,700 ng mL h^{-1} and 4710 ng mL h^{-1}; C_{max} = 1490 ng mL^{-1} and 340 ng mL^{-1} in rat and dog, respectively) and half-life ($T_{1/2}$ = 22 h and 8 h in rat and dog, respectively). These results suggest the potential advantage of using a symmetrical pyrimidine moiety, such as that found in compound **viii** and SCH-D, in place of the unsymmetrical pyridine N-oxide of compound **vii** and SCH-C.

5 Ross, T. *et al.* (1999) Role of chemokine receptors in HIV-1 infection and pathogenesis *Adv. Virus Res.* 52, 233–267

6 Liu, R. *et al.* (1996) Homozygous defect in HIV-1 co-receptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 86, 367–377

7 McCombie, S. W. *et al.* (2003) Piperazine-based CCR5 antagonists as HIV-1 inhibitors. III: synthesis, antiviral and pharmacokinetic profiles of symmetrical heteroaryl carboxamides *Bioorg. Med. Chem. Lett.* 13, 567–571

8 Tagat, J. R. *et al.* (2001) Piperazine-based CCR5 antagonists as HIV-1 inhibitors. II. Discovery of 1-[2,4-dimethyl-3-pyridinyl)carbonyl]-4-methyl-4-[3(*S*)-methyl-4-[1(*S*)-[4-(trifluoromethyl)phenyl]ethyl-1-piperazinyl]-piperidine N1-oxide (Sch-350634), an orally bioavailable, potent CCR5 antagonist. *J. Med. Chem.* 44, 3343–3346

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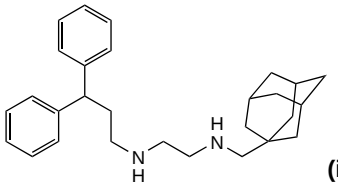
Molecules

Antituberculosis agents

Tuberculosis (TB) is the primary cause of human deaths that are attributable to a single etiologic agent, with almost three million deaths per year a result of infection with *tubercule bacillus*. Current chemotherapy consists of two phases: an intensive two-month period of daily therapy, followed by a four-month continuation phase. In most patients, sputum is cleared of live bacteria within two months of commencing oral therapy, but the full six-month course is required to prevent relapse after therapy is discontinued. Many decades of poor patient compliance with this prolonged and complex regimen has had two consequences: first, treatment of the disease is often unsuccessful and, second, there is an expanding epidemic of drug resistance that threatens TB control programs worldwide. One route that could decrease the length of treatment would be to improve the potency of the current anti-tuberculosis agents. This strategy would enable higher effective dosing of patients and could improve the therapeutic effect of the agent by maintaining the drug concentration above the minimum inhibitory concentration (MIC) for longer periods of time, or by enhancing the ratio of the peak:trough concentration to the MIC.

Drugs that affect the cell wall of the bacteria are known to have concentration-dependent cidal effects *in vitro* that might be achievable *in vivo* with enhanced potency. In an effort to discover more clinically effective treatments for *Mycobacterium tuberculosis*, the high throughput synthesis of libraries of potential inhibitors was undertaken [1]. Several large libraries of compounds were synthesised in mixtures of ten on rink acid resin (Novabiochem; http://www.novabiochem.com). A total of 63,238 compounds were synthesised and, from this set, several active mixtures were identified. Some of these mixtures were deconvoluted and the activity of single compounds was determined in an assay of the minimum inhibitory concentration, and a bioluminescent reporter strain assay that produces light in response to inhibition of cell wall synthesis by ethambutol. Several potent analogues were obtained upon deconvolution, with compound **i** being one of the most potent, possessing an MIC value of 0.5 mM. This work has delivered inhibitors with many unique structural features that suggest further routes for optimisation and, thus, further work in this area is warranted.



(i)

1 Lee, R. E. *et. al.* (2003) Combinatorial lead optimisation of [1,2]-diamines based on ethambutol as potential antituberculosis preclinical candidates. *J. Combi. Chem.*, 5, 172–187

Piperidines targeting the nociceptin receptor

The nociceptin/orphanin FQ (N/OFQ) receptor (NOP, previously named ORL-1) was discovered by several research groups in 1994, through cDNA expression cloning techniques. Its endogenous ligand nociceptin (N/OFQ), a novel heptadeca neuropeptide, was subsequently isolated from brain and identified in 1995. This discovery generated considerable interest, due to the important role of classical opioid receptors in the CNS. Although the NOP receptor is a member of the G-protein coupled receptor superfamily, with about 47% identity to the classical opioid receptors [MOP (μ), DOP(δ) and KOP (κ)], native opioid peptides and synthetic agonists that are selective for MOP, DOP or KOP receptors do not show significant affinity for the NOP receptor. Using nociceptin (N/OFQ) and its peptide analogues, a number of *in vivo* experiments have demonstrated that N/OFQ modulates a variety of

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