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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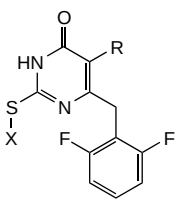
Antiviral molecules

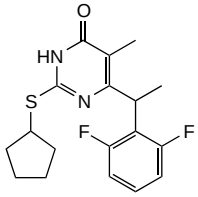
Conformationally restricted NNRTIs

The structurally diverse HIV non-nucleotide reverse transcriptase inhibitors (NNRTIs) share a common mechanism of action; that is, binding to an allosteric site close to the active site of the enzyme and inhibiting transcription. 5-Alkyl-2-(alkylthio)-6-(2,6-difluorobenzyl)-3,4-dihydropyrimidine-4(3H)-ones [S-DABOs; **(i)**] are a recently identified class of NNRTI that is capable of inhibiting HIV at nanomolar concentrations[1].

In a recent follow-up study, Mai and coworkers[2] investigated conformationally restricted analogues of compound **(i)** (X = alkyl or cycloalkyl). Rather than forming an additional ring, conformational control in this series was achieved by introducing methyl groups at the C5 position of the pyrimidine and at the benzylic carbon bearing the 2,6-difluorophenyl group. The addition of these two groups greatly restricts the rotational freedom of the benzylic group, locking it into the preferred conformation for binding to HIV reverse transcriptase. The most active compound in this series is **(ii)** (EC_{50} = 6 nM, CC_{50} >200 μM). Further SAR studies on enantiomerically pure material indicated that the stereochemistry of the benzylic center is crucial, with the (+)-isomer (EC_{50} = 2 nM) being more active than the (–)-isomer (EC_{50} = 70 nM). The 2,6-difluorophenyl group is a preferred substituent that is consistent with a putative charge-transfer interaction with the Tyr188 residue of the enzyme.



(i)



(ii)

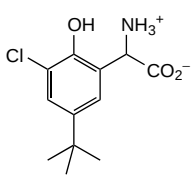
1 Mai, A. *et al.* (1999) 5-Alkyl-2-(alkylthio)-6-(2,6-dihalophenylmethyl)-3,4-dihydropyrimidin-4(3H)-ones: novel potent and selective dihydro-alkoxy-benzyl-oxopyrimidine derivatives. *J. Med. Chem.* 42, 619–627

2 Mai, A. *et al.* (2001) Structure-based design, synthesis, and biological evaluation of conformationally restricted novel 2-alkylthio-6-[1-(2,6-difluorophenyl)alkyl]-3,4-dihydro-5-alkylpyrimidin-4(3H)-ones as non-nucleoside inhibitors of HIV-1 reverse transcriptase. *J. Med. Chem.* 44, 2544–2554

Amino-acid derived inhibitors of influenza neuraminidase

Virally expressed neuraminidase is an essential enzyme in the life cycle of the influenza virus. The enzyme is present on the outer surface of the virus and cleaves *N*-acetylneuraminic acid residues from cellular glycoproteins, glycolipids and oligosaccharides. This enables the nascent virus to diffuse freely and infect neighboring cells.

A recent paper from researchers at Abbott Laboratories (Abbott Park, IL, USA) describes the discovery of non-carbohydrate inhibitors of influenza neuraminidase[3]. This is significant because current inhibitors of this enzyme are derived from carbohydrate or carbohydrate-mimetic templates. Rationalizing that an α- or β-amino acid could interact with enzyme residues Asp152 and Tyr406, which are located in the active site of neuraminidase, a collection of 300 such compounds was screened. This resulted in the discovery of a phenylglycine derivative **(iii)** and a pyrrolidine derivative **(iv)**.



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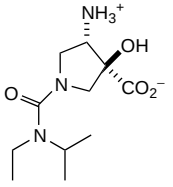


(iv)

The measured binding constants for **(iii)** and **(iv)** are 41 μM and 0.36 μM, respectively, against neuraminidase derived from an influenza A strain. X-ray structures of both classes of inhibitors bound to neuraminidase were determined[3]. Interestingly, the X-ray structure of a pyrrolidine-based inhibitor showed a previously unobserved interaction with residue Tyr406 of the enzyme, which is significant because this residue is absolutely conserved across all strains of the virus.

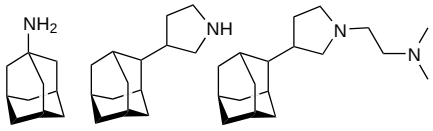
1359-6446/01/$ – see front matter PII: S1359-6446(01)02040-2

3 Kati, W.M. *et al.* (2001) Novel α- and β-amino acid inhibitors of influenza virus neuraminidase. *Antimicrob. Agents Chemother.* 45, 2563–2570

New adamantyl-based anti-influenza A agents

Amantadine **(v)** has long been known as an antiviral agent active against influenza A and is believed to act as a blocker of the M2 ion-channel of influenza A (Ref. 4). Despite the effectiveness of this compound it is only active against the A strain of the virus, not the B strain, and resistance mutations arise quickly during treatment. Research efforts to develop improved versions of this drug are under way in several laboratories.

Cyclic pyrrolidine-based adamantanes, such as **(vi)** and **(vii)**, have recently been reported[5]. These compounds were found to be active in preventing virus-induced cytopathogenicity in influenza A infected MDBK (Madin–Darby bovine kidney) cells. It is worth noting that dialkylaminoethyl substitution of amantadine yields inactive compounds but that the analogous substitution in the pyrrolidine series is tolerated.



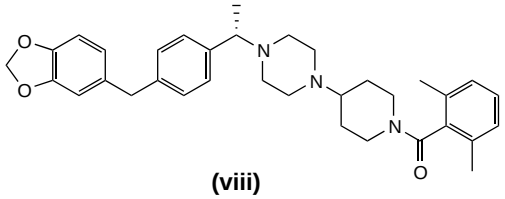
(v) (vi) (vii)

4 Hay, A.J. *et al.* (1985) The molecular basis of the specific anti-influenza action of amantadine. *EMBO J.* 4, 3021–3024

5 Stamatiou, G. *et al.* (2001) Novel 3-(2-adamantyl)pyrrolidines with potent activity against influenza A virus-identification of aminoadamantane derivatives bearing two pharmacophoric groups. *Bioorg. Med. Chem. Lett.* 11, 2137–2142

CCR5 antagonists as HIV-1 inhibitors

Viral entry is an emerging target in the fight against HIV infection. Recently, it was shown that viral binding to the chemokine receptors CD4 and CCR5 was required for entry by macrophage (M)-tropic strains of the virus[6]. Fortunately, it was found that HIV-binding to the CCR5 co-receptor, and thus viral entry, could be inhibited by the endogenous ligands for this receptor, RANTES (regulation upon-activation, normal T-cell expressed and secreted) and the macrophage inflammatory proteins, MIP-1α and MIP-1β. These observations suggested that small-molecule antagonists of the CCR5 receptor might be useful as anti-HIV agents.



(viii)

Recently, an example of this approach was disclosed by Tagat and coworkers[7] from the laboratories of Schering-Plough (Kenilworth, NJ, USA). For example, compound **(viii)** was found to inhibit the binding of RANTES to the CCR5 receptor *in vitro* (K_i = 31 nM). In cell culture it inhibited HIV-1 entry with an IC_{50} value of 1.7 nM. Furthermore, when tested against a primary HIV-1 isolate (US-1), compound **(viii)** inhibited viral replication in peripheral blood mononuclear cells with a mean IC_{50} value of 8 nM.

6 Littman, D.R. (1998) Chemokine receptors: keys to AIDS pathogenesis? *Cell* 93, 677–680

7 Tagat, J.R. *et al.* (2001) Piperazine-based CCR5 antagonists as HIV-1 inhibitors I: 2(*S*)-methyl piperazine as a key pharmacophore element. *Bioorg. Med. Chem. Lett.* 11, 2143–2146

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Drug delivery and drug targeting

Drug targeting using thermally responsive polymers and local hyperthermia

There is an ongoing need for drug delivery methods that are aimed at targeting cancer chemotherapeutics to tumors. Many cytotoxic chemotherapeutics are equally as toxic to healthy tissues, so specific targeting to tumor cells is a great advantage. Attaching these drugs to soluble polymeric drug carriers improves drug pharmacokinetics, and leads to increased accumulation of the drug at the tumor site over free drug because of passive targeting, an effect that is referred to as the enhanced permeability and retention (EPR) effect. Although this can be an advantage, polymeric carriers do not target a specific site. The tumor cytotoxicity of chemotherapy or radiotherapy is enhanced synergistically by the application of hyperthermia[1,2]. Hyperthermia preferentially increases the permeability of tumor vasculature over normal vasculature, which can further enhance preferential delivery to tumors. Thermal targeting of polymeric drug carriers could offer synergistic advantages over either technique used alone.

Synergism studies

Meyer and coworkers have recently reported the use of two thermally responsive polymeric drug carriers to target tumors[3]. Their working hypothesis was that polymeric drug carriers that undergo a lower critical solution temperature (LCST) phase transition could be designed so they remain in solution *in vivo* after systemic injection, until they reach a tumor that is locally heated above the LCST. The temperature of the LCST was chosen at 40°C, because this is higher than the physiological body temperature (37°C) but lower than 42°C, a temperature that is regularly used for hyperthermia treatments in cancer patients. Using rhodamine as a model compound, they examined the effects that an LCST transition had on

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