Monitor: molecules and profiles

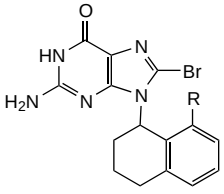
Monitor provides an insight into the latest developments in drug discovery through brief synopses of recent presentations and publications together with expert commentaries on the latest technologies. There are two sections: *Molecules* summarizes the chemistry and the pharmacological significance and 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

Guanine analogues as phosphodiesterase 7 (PDE7) inhibitors

The secondary messengers cAMP and cGMP are regulated by phosphodiesterases (PDEs), which hydrolyze them to the corresponding inactive 5′-monophosphate nucleotides. To date, 11 PDE gene families have been identified, varying in substrate specificity, inhibitor sensitivity and regulatory characteristics[1]. In particular, PDE7 is a cAMP-specific enzyme with a low K_m (0.2 μM), which is insensitive to the standard PDE4 inhibitor, rolipram[2]. The PDE7 mRNA is widely distributed, although the active protein has been identified predominantly in T-cells. It is suggested that selective inhibitors of PDE7 could have benefits in the treatment of T-cell-mediated diseases. In addition, the presence of PDE7 in airway epithelial cells implies that inhibitors could be beneficial in airway disease therapy.

To date, only two series of synthetic PED7 inhibitors have been described[3], which lack selectivity over the PDE4 and PDE3 isozymes. Because this could result in several side effects (e.g. emesis and cardiotoxicity), selective PDE7 inhibitors are highly desired. In a recent paper[4], Davenport and coworkers report on a series of guanine analogues that possess PDE7 inhibitory activity *in vitro* and demonstrate some evidence of selectivity over PDE4 and PDE3 isoenzymes. Their initial guanine-based hit [compound **(i)**; R = H] was identified as a result of screening internal and external databases. Extended SAR studies on compound **(i)** showed that the removal of the bromine and the amino group, as well as the replacement of the saturated six-membered ring with a five-membered and a seven-membered ring, reduced its activity. On the contrary, when a substituent (e.g. bromine, methoxy or nitro group) was inserted into the tetralin ring, improved activity was seen. In particular, compound **(ii)** (R = Br) was the most potent (IC_{50} = 1.31 μM) and selective (<14% inhibition for PDE3 and PDE4 at 10 μM).



(i) R = H
(ii) R = Br

Finally, because the natural ligand for PDE7 contains an adenine base, several compounds, in which the guanine was substituted with an adenine, were prepared. However, none of these analogues was found to offer any advantage over compound **(ii)**.

1 Rascon, A. (1997) Cyclic nucleotide phosphodiesterases: diversity, classification, structure and function. *Acta Cient. Venez.* 48, 145–153

2 Kaulen, P. *et al.* (1989) Autoradiographic mapping of a selective cyclic adenosine monophosphate phosphodiesterase in rat brain with the anti-depressant [^{3}H] rolipram. *Brain Res.* 503, 229–245

3 Martinez, I. *et al.* (2000) Benzyl derivatives of 2,1,3-benzo- and benzothieno[3,2-a]thiadiazine 2,2-dioxides: first phosphodiesterase 7 inhibitors. *J. Med. Chem.* 43, 683–689

4 Barnes, M.J. (2001) Synthesis and structure-activity relationships of guanine analogues as phosphodiesterase 7 (PDE7) inhibitors. *J. Med. Chem.* 11, 1081–1083

Daniela Barlocco
University of Milan,
Viale Abruzzi 42
Milano-20131, Italy
tel: +39 02 2950 2223,
fax: +39 02 2951 4197
e-mail: daniela.barlocco@unimi.it

Novel antiviral molecules

Anti-HIV activity of betulinic acid analogue YKFH312

Highly active antiretroviral therapy (HAART) has proven to be beneficial in the treatment of HIV, however, it has become increasingly clear that the therapy needs to be improved. One of the principal problems has been the emergence of viral resistance and several approaches are being pursued to slow down or overcome this resistance. One approach is to discover agents that attack viral targets, other than HIV polymerase and HIV protease, which form the basis of existing

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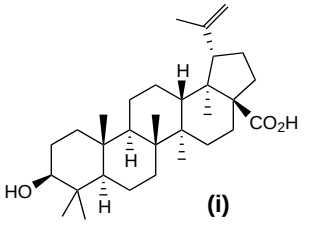
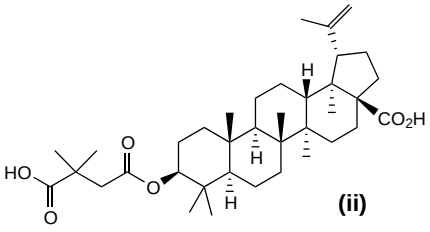
therapies. A rich source of compounds with unique mechanisms of action has been the natural products.

Betulinic acid **(i)**, a natural product isolated from *Syzigium claviforum*, shows potent antiviral activity against HIV in MT-4 cells in a cell protection assay. Likewise, synthetic derivatives were found to be active, with compound **(ii)** (YKFH312) being the most potent analogue synthesized (having an EC_{50} value of 0.011 μg ml^{-1}). The mechanism of this chemotype is reported by Kanamoto and coworkers[1]. Additional cell culture assays revealed that YKFH312 did not inhibit integration or pre-integration events in the viral life-cycle. Viral protein production and expression on the cell surface were not inhibited, although the compound did inhibit the release of infectious virus particles from persistently infected MOLT-4/HIV-1_{IIIB} cells. *In vitro* experiments indicated that YKFH312 was not an inhibitor of HIV polymerase or HIV protease. The conclusion from this study is that YKFH312 interferes with viral maturation by a mechanism that is unique from currently approved therapeutic agents, although the exact target is still not known. As such, YKFH312 presents a promising opportunity to develop a new antiretroviral agent to add to the current therapeutic cocktail.



(i)

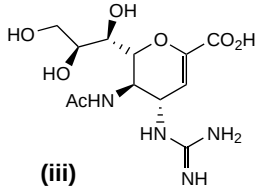


(ii)

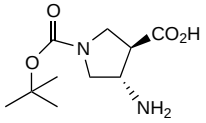
1 Kanamoto, T. *et al.* (2001) Anti-human immunodeficiency virus activity of YK-FH312 (a betulininc acid derivative), a novel compound blocking viral maturation. *Antimicrob. Agents Chemother.* 45, 1225–1230

New inhibitors of influenza neuraminidase

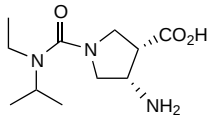
Influenza kills 20,000–40,000 people annually in the USA alone and past pandemics, such as that which occurred in 1918, were even more devastating. Despite this, there are currently only limited therapeutics available to those who become infected, including the inhibitors of influenza neuraminidase, such as zanamivir **(iii)** (Ref. 2). A new entry into this area has been recently disclosed by a group from Abbott Pharmaceuticals (Abbott Park, IL, USA)[3]. This group's work is based on the interesting discovery that the pyrrolidine-based compound A87380 **(iv)** yields modest activity against influenza neuraminidase (with an IC_{50} value of 50 μM) derived from the A/Tokyo (influenza A) strain. Derivatization of the endocyclic nitrogen through combinatorial chemistry yielded compound **(v)** which has improved potency (IC_{50} = 1.6 μM). It is known that each of the four substituents of zanamivir occupies a specific pocket in the enzyme active site, so it was reasoned that the addition of a fourth substituent to **(v)** would result in a further improvement in activity. Indeed, the methyl-trifluoroacetamido modified analogue **(vi)** was found to have an IC_{50} value of 0.28 μM, which is almost a tenfold increase in activity.



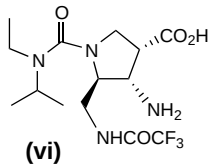
(iii)



(iv)



(v)



(vi)

2 Hayden, F.G. *et al.* (1997) Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infection. *N. Engl. J. Med.* 337, 874–880

3 Wang, G.T. *et al.* (2001) Design, synthesis, and structural analysis of influenza neuraminidase inhibitors containing pyrrolidine cores. *J. Med. Chem.* 44, 1192–1201

Isoprenylation inhibitors as antiviral agents

The plasma-membrane attached protein, RhoA, has been suggested to have a role in viral entry for respiratory syncitial virus (RSV), because of its interaction with the viral fusion glycoprotein (F). Moreover, RhoA is anchored to the plasma membrane through a geranylgeranyl group attached to the C-terminus of the protein. This has led a research group at Vanderbilt University (Nashville, TN, USA) to investigate whether inhibitors of protein isoprenylation could indirectly inhibit RSV entry by reducing the plasma-membrane concentration of RhoA (Ref. 4).

To this end, the group chose to look at the effect of the 3-hydroxy-3-methylglutaryl (HMG)-CoA inhibitor, lovastatin, on RSV replication both *in vivo* and in cell-culture assays. In addition to inhibiting HMG-CoA and reducing serum cholesterol levels, lovastatin also has many other effects on cells including inhibiting the production of isoprenyl groups on RhoA. This could conceivably inhibit the geranylgeranylation of RhoA, and result in the drug acting as an antiviral agent. Although this specific mechanism is not addressed directly by the authors, several experiments were conducted that suggest this drug does have an effect on RSV replication *in vivo*. For example, mice treated with lovastatin (1 mg per day), beginning three days before infection with RSV, show a 1–2 log_{10}

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reduction in viral titer compared with untreated mice. In addition, treatment with an unrelated cholesterol-reducing agent had no antiviral effect and lovastatin had no antiviral effect on vaccinia virus. Finally, no reduction in serum cholesterol was observed during the time the mice were treated.

These results are promising and suggest that an already approved class of drugs could be used in the battle against certain viral diseases, such as RSV. It is hoped that future experiments will determine if lovastatin does act by reducing the plasma membrane expression of RhoA.

4 Gower, T.L. and Graham, B.S. (2001) Antiviral activity of lovastatin against respiratory synceytial virus *in vivo* and *in vitro*. *Antimicrob. Agents Chemother.* 45, 1231–1237

PARP-1 as a potential anti-HIV target

Infection by HIV-1 requires the integration of a DNA copy of the viral genome into the host DNA. Integration is achieved by a multi-step mechanism involving processing of the 3′-ends of double stranded viral DNA by clipping off two nucleotides and inserting the processed ends into the host genome. This pathway leads to an intermediate with gaps at either end of the integrated DNA. These gaps are repaired by an enzyme, or enzymes, which have yet to be identified. A recent publication[5] by researchers from John Hopkins University (Baltimore, MD, USA) suggests that one of the enzymes involved in gap repair is PARP-1 [poly(ADP-ribose) polymerase-1; EC2.4.2.3.0], a host enzyme. PARP-1 is a nuclear enzyme that is activated by DNA strand breaks and, as such, might be activated by the integration intermediate already described. To test this hypothesis, the John Hopkins team looked at the infection of fibroblasts isolated from PARP-1 knockout mice by a pseudotyped HIV virus. As expected, the virus infected normal fibroblasts (PARP-$1^{+/+}$) but not the knockout fibroblasts (PARP-$1^{-/-}$). If PARP-1 is involved in HIV integration, as these results suggest, then PARP inhibitors might be useful in treating HIV-1 infection.

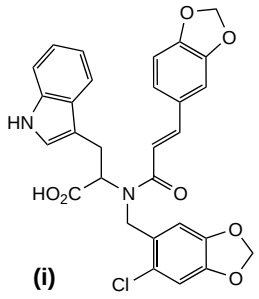
5 Ha, H.C. *et al.* (2001) Poly(ADP-ribose) polymerase-1 is required for efficient HIV-1 integration. *Proc. Natl. Acad. Sci. U. S. A.* 98, 3364–3368

Michael A. Walker
Bristol-Myers Squibb
Pharmaceutical Research Institute
Wallingford
CT 06492, USA
tel: +1 203 677 6686
fax: +1 203 677 7702
e-mail: walkerma@bms.com

Combinatorial chemistry

Endothelin receptor antagonists

Endothelin (ET-1) is a potent vasoconstrictor consisting of 21 amino acids. ET-1, as well as two other structurally and functionally related vasoconstricting peptides (endothelin-2 and endothelin-3), interacts with two known G-protein coupled receptors (ET_A and ET_B) and induces vasoconstricting effects. ET_A, ET_B and the more recently discovered ET_C, are tissue specific and displayed preferentially in varying proportions on different cell types. ET_A is found in vascular smooth-muscle tissue and is mainly responsible for vasoconstriction of smooth muscle cells, whereas ET_B, which is found in nonvascular smooth-muscle tissues, has been implicated in the release of endothelin-derived relaxing factors. Elevated levels of endothelin are found in patients suffering from a variety of diseases, including hypertension, pulmonary hypertension and cerebral vasospasm, and evidence is accumulating that newly discovered ET antagonists could not only provide a novel therapy for the treatment of such patients but also help in understanding the precise physiological roles of endothelins. In an effort to discover new ET antagonists, a library of 15 individual peptoid compounds was synthesized[1]. Screening of these compounds revealed their affinity for endothelin receptors in terms of their ability to competitively inhibit ET. One of the most potent compounds discovered was **(i)**, which possessed an IC_{50} of 660 nM against ET_B and 13-fold selectivity over ET_A. This library has been successful in providing novel peptoids possessing a range of ET_A and ET_B receptor affinities. Future work could be directed at comparing biophore mapping of this series with the energy-minimized three-dimensional model of other active compounds from the literature, which could aid in the design of further compounds with improved activity.



(i)

1 Dasgupta, F. *et al.* (2001) Peptoids as endothelin receptor antagonists. *Bioorg. Med. Chem. Lett.* 11, 555–557

Dopamine D_4 receptor partial agonists

Recent advances in molecular cloning techniques have led to the characterization of several dopamine receptor subtypes, which can be divided into the D_1- and D_2-like families. The D_1-like family comprises the D_1 and D_5 subtype, whereas the D_2-like family consists of the D_2, D_3 and D_4 receptors. Because of the preferred expression of mRNA for dopamine receptors in the frontal cortical and mesolimbic areas, considerable interest has been focused on selective D_4 antagonists. According to recent neuropathological and genetic studies[2] selective dopamine D_4 receptor agonists, partial agonists or antagonists, might be of interest for the treatment of neuropsychiatric disorders including attention-deficient hyperactivity, mood disorders and Parkinson's disease[3].

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