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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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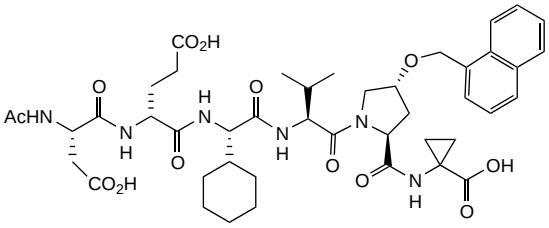
Molecules

Hepatitis C virus protease-inhibitors

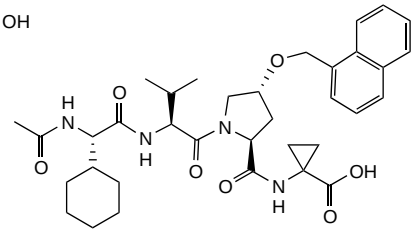
Hepatitis C virus (HCV) infection has recently emerged as a significant disease and has, therefore, become the focus of extensive discovery efforts. Research in this area has previously been hindered by an inability to grow the virus in tissue culture. However, current efforts have focused on screening for inhibitors of viral enzymes encoded by the HCV genome. One of the most widely studied targets is the viral serine-protease located at the NS3 domain of the viral genome (i.e. the NS3/4A protease). This enzyme is responsible for processing the non-structural portion of the translated viral polyprotein and is essential for virus replication. Additional factors that make this protease an attractive target are the recent clinical success of HIV-protease inhibitors and the extensive knowledge regarding serine proteases.

A group at Boehringer Ingelheim (Laval, Quebec, Canada) have discovered that the NS3/4A protease is susceptible to inhibition by a hexapeptide, Asp-Asp-Ile-Val-Pro-Cys [IC_{50} = 71 μM], that corresponds to the P_6–P_1 residues of one of its native substrates. A recent publication from this group describes their efforts towards increasing the binding affinity of this inhibitor to the enzyme, while reducing the size of the inhibitor[1]. Optimization of the amino acid side-chains of the peptide generated hexapeptide **(i)** (IC_{50} = 0.013 μM), which contains modified P_1, P_2, P_4 and P_5 residues. Interestingly, the SAR of the P_2 side-chain demonstrated that large aromatic groups yield stronger binding. In addition, the cyclopropyl ring at P_1 represents a rather unique replacement for the cysteine residue found in the substrate. More importantly, this peptide provides a good starting point for trimming the size of the inhibitor. Pruning the P_6 and P_5 residues from **(i)** yields tripeptide **(ii)**, (IC_{50} = 3.5 μM), which is smaller and more active than the parent inhibitor.

Researchers at the Instituto di Richerche di Biologia Molecolare (IRBM; Pomeza, Rome, Italy) have recently disclosed a substrate-based peptide HCV-protease inhibitor (IC_{50} = <0.2 nM)[2]. However, in contrast to the Boehringer group, peptide **(iii)** includes residues P_6–P_4'. The design of this inhibitor follows the observation that an *N*-methylated P_1 (or P_1') or cyclic P_1' residue leads to a non-cleavable substrate. This approach is of particular interest, because it has been recognized that the majority of the ground-state binding affinity of the HCV-protease comes from its S-subsite (carboxy-binding) domain, and that the C-terminal carboxylate makes a significant contribution to the binding of product-based inhibitors. However, the crystal structure of the protease reveals several potential binding pockets in the S′-domain (amino-binding domain). Peptide **(iii)** was derived from a P′-subsite (amine-containing portion) optimized using combinatorial chemistry in the context of a potent P-subsite (carboxylic acid portion) sequence.



(i)



(ii)

Ac-Asp-D-Glu-Leu-Ile-Cha*-Cys-Pro-Cha-Asp-Leu-NH_2
IC_{50} <0.2 nM
*(Cha = β-cyclohexyl-alanine)

(iii)

 PII: S1359-6446(01)01702-0

1 Llinàs-Brunet, M. *et al.* (2000) Highly potent and selective peptide-based inhibitors of the hepatitis C virus serine protease: towards smaller inhibitors. *Bioorg. Med. Chem. Lett.* 10, 2267
2 Ingallinella, P. *et al.* (2000) Optimization of the P′-region of peptide inhibitors of hepatitis C virus NS3/4A protease. *Biochemistry* 36, 12898

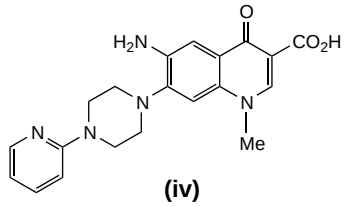
Viral chemokine inhibitor of HIV

Cellular entry of HIV via binding to the chemokine receptors CCR5 or CXCR4 is well established. It is also known that this process can be inhibited by the endogenous ligands for these receptors, such as RANTES (regulated on activation normal T-cell expressed and secreted) and macrophage inflammatory protein-1β (ligands for CCR5) and stromal cell-derived factor-1α (ligands for CXCR4). Interestingly, a viral chemokine, viral macrophage inflammatory protein II [vMIP-II], which is encoded by the herpes 8 virus, is also capable of binding to these receptors. Recent SAR studies by researchers at Thomas Jefferson University (Philadelphia, PA, USA) revealed that trimming the peptide back to the N-terminal domain (residues 1–21) yields a selective inhibitor for the CXCR4 receptor (IC_{50} = 0.64 μM)[3], and demonstrated that replacement of ^{11}Cys with Ala, Gly or Phe resulted in an increase in binding affinity. Furthermore, the peptide was able to inhibit viral replication, cytopathicity and syncytia formation at a concentration of ~50–100 μM.

3 Luo, Z. *et al.* (2000) Structure–function study and anti-HIV activity of synthetic peptide analogues derived from viral chemokine vMIP-II. *Biochemistry* 39, 13545

6-Aminoquinolones as anti-HIV agents

The search for new anti-HIV therapeutics has resulted in the discovery of a new chemotype from a group of quinolone antibacterial compounds and, furthermore, this research has identified a previously unexploited target, viral RNA. Researchers at the University of Perugia (Perugia, Italy) and the University of Padova (Padova, Italy) report preliminary SAR studies of this class of molecule[4]. Thus, compound **(iv)** was found to be a potent (EC_{50} = 0.1 μM), but mildly cytotoxic (TC_{50} = 7 μM), antiviral compound selective for HIV in cell culture (C8166 cells). The mechanism of this inhibition is not yet known, but appears to be associated with binding to the *Tar* RNA of HIV.



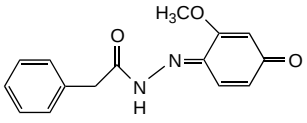
(iv)

4 Cecchetti, V. *et al.* (2000) 6-Aminoquinolones as new potent anti-HIV agents. *J. Med. Chem.* 43, 3799

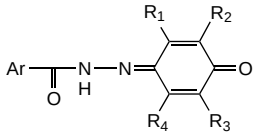
Novel potential enhancers of nerve growth factor

Nerve growth factor (NGF) is a polypeptide that acts as a prototypical neurotrophic factor essential for the growth and development of neurons in the central and peripheral nervous systems. From experimental data it is hypothesized that NGF could be effective in the treatment of dementia and cerebral paralysis. However, the compound can only be administered intraventricularly, because it does not cross the blood–brain barrier. On this basis, attempts are being made to identify low MW compounds with neurotrophic action.

The fungal metabolite NG061 **(v)**, was isolated by a Japanese group[5] from the fermentation broth of *Penicillium minioluteum* F-4627, and appeared to be an appropriate substrate for further investigation as a potential enhancer of NGF activity. Recently, the same group reported the synthesis of **(v)** and several analogs [**(v)**a–y], comprising every possible combination of R and Ar groups: hydrogen, alkyl, halogen, benzyl and substituted phenyl[6]. As previously reported for **(v)**, the new synthetic compounds should be in equilibrium in solution when obtained from asymmetrical quinones, as a result of the presence of geometrical isomers.



(v)



(v) a–y

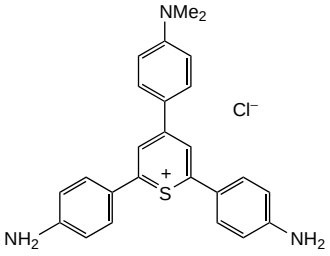
These compounds were tested for their ability to enhance the effect of NGF on neurite outgrowth in PC12 cells. Several derivatives were highly cytotoxic at 10 μg ml^{-1}, and, therefore, were tested no further. The synthesized compound **(v)** had the same effect on neurite outgrowth as NGF, whereas its analogues were less potent. In particular, substitution of the phenylacetyl moiety with an acetyl group significantly reduced NGF-like activity, and replacement of the methoxy group either with hydrogen or an alkyl group was also detrimental. Furthermore, the di-*tert*-butyl derivatives inhibited the effect of NGF. However, the ease of synthesis of NG061 and its derivatives could provide novel enhancers of NGF activity.

5 Bhandari, R. *et al.* (1999) Structure of NG061, a novel potentiator of nerve growth factor (NGF) isolated from *Penicillium minioluteum* F-4627. *J. Antibiot.* 52, 231–234
6 Eguchi, T. *et al.* (2000) Synthesis of NG061 and its analogs, and their biological evaluation as enhancers of nerve growth factor. *Chem. Pharm. Bull.* 48, 1470–1473

A selenopyrylium photosensitizer for photodynamic therapy

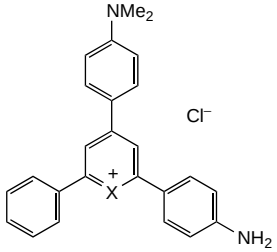
Photodynamic therapy (PDT) is a recently approved protocol for the treatment of cancer that combines light and endogenous oxygen with a photosensitizer localized in or around the tumour. However, the photosensitizing agent Photofrin®, which has approval for clinical use in PDT against several tumour types, has disadvantages. This has driven the search for other porphyrin and porphyrin-like

materials as sensitizers for use in PDT. In addition, various classes of cationic dyes have been explored as sensitizers. These materials typically have absorption maxima values of >600 nm and have molar extinction coefficients of $\geq 10^4$ M cm^{-1}, which make them more effective than Photofrin® and other porphyrin-derived sensitizers[7]. The compound 2,6-*bis*(4-aminophenyl)-4-(dimethylaminophenyl) thiopyrylium chloride [AA1,**(vi)**], selectively accumulates in tumours, but it has an absorption maxima value of <600 nm and low quantum yield for the generation of singlet oxygen [$\Phi(^1O_2) = <0.01$], which is a cytotoxic species generated by irradiation of the sensitizer.



(vi)

On the basis of their previous results in this field, Detty and coworkers have recently reported[8] a series of chalcogenopyrylium dyes [**(vii)–(ix)**], structurally related to **(vi)**. In particular, compound **(viii)** (X = Se), when tested *in vivo* against R3230AC mammary adenocarcinomas in female Fischer rats, demonstrated a 300% increase in tumour-doubling time compared with untreated control animals.



(vii) X = S
(viii) X = Se
(ix) X = Te

Furthermore, because of its relatively low log-P value (0.8), no skin photosensitivity was observed in treated animals. The authors suggest that the singlet-oxygen-induced damage to mitochondria [$\Phi(^1O_2) = 0.029 \pm 0.005$ in methanol], is a possible mechanism of action.

Novel derivatives of this class, which retain one anilino substituent and one 4-dimethylanilino substituent while the third substituent is varied, are being studied with the aim of optimizing their properties for PDT.

7 Dougherty, T.J. *et al.* (1998) Photodynamic therapy. *J. Natl. Cancer Inst.* 90, 889–905

8 Detty, M.R. *et al.* (2000) A selenopyrylium photosensitizer for photodynamic therapy related in structure to the antitumor agent AA1 with potent *in vivo* activity and no long-term skin photosensitization. *J. Med. Chem.* 43, 4488–4498

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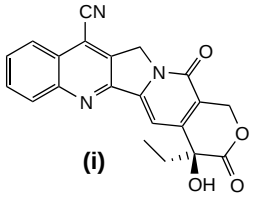
Novel antitumour molecules

Novel substituted camptothecins with potent antitumour activity

Inhibition of the enzyme DNA topoisomerase I, which is essential for the relaxation of DNA structure during several crucial cellular processes, has proven to be an attractive strategy in anticancer drug design. Two drugs in this class, Camptosar® (CPT11) and Hycamtin® (topotecan), which are related to the alkaloid camptothecin, have received FDA approval for use in the treatment of certain types of solid tumours, and intensive efforts to find new topo-I inhibitors are continuing. Two reports of new camptothecin analogues with potent anticancer activity have recently been described that have focused on analogues with differing substituents in the A/B rings, which modelling studies have suggested would optimize inhibition. Merlini and coworkers have reported the synthesis and evaluation of new camptothecins substituted in position 7 with alkyl or alkenyl chains bearing cyano and/or carboethoxy groups[1]. Most notably, the 7-cyano-camptothecin analogue **(i)** demonstrated high *in vitro* cytotoxicity against a topotecan-resistant H460 (non-small-cell lung cancer) cell line and a cisplatin-resistant ovarian carcinoma cell line. *In vivo* evaluation indicated that **(i)** was more cytotoxic than topotecan in the H460 tumour model, and was comparable with topotecan in both a small-cell lung carcinoma model and a colon carcinoma model; hence, further preclinical evaluation of this compound would be desirable.



(i)

Because camptothecins contain an α-hydroxy-δ-lactone they exist in two distinct forms at physiological pH: a biologically active 'lactone-closed' form and a biologically inactive 'lactone-opened' form. Hydrolysis to generate the lactone-opened form inactivates the parent drug, a problem that is exacerbated in human blood because the abundant blood-serum protein, albumin, preferentially binds to this form. Establishing the physiological conditions required to achieve a therapeutically relevant concentration of the lactone-closed form in tumour cells, therefore, is a major challenge in this field. This has been addressed by Burke and coworkers, who have described a novel silatecan **(ii)**, which has a closed lactone ring and displays high lipophilicity, improved human blood stability and potent anticancer activity[2]. The combination of its potency and stability profiles suggests that **(ii)** could be more efficacious than the currently used camptothecin-based therapies.

1359-6446/01/$ – see front matter PII: 1359-6446(01)01703-2