Monitor

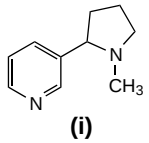
Monitor provides an insight into the latest developments in the pharmaceutical and biotechnology industries. ***Chemistry*** examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while ***Profiles*** provides commentaries on promising lines of research, new molecular targets and technologies. ***Biology*** reports on new significant breakthroughs in the field of biology and their relevance to drug discovery. ***Business*** reports on the latest patents and collaborations, and ***People*** provides information on the most recent personnel changes within the drug discovery industry.

Chemistry

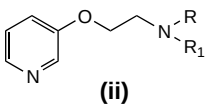
Molecules

Aminobutinylpyridines as ligands for the $\alpha_4\beta_2$ nicotinic cholinergic receptors

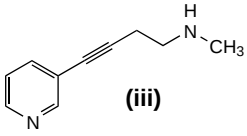
Several populations of nicotinic acetylcholine (nACh) receptors have been described. The $\alpha_4\beta_2$ subtype seems to be prevalent in mammalian brain [1]. In addition to the natural ligand nicotine (**i**), novel compounds with 'extended' side chains have been shown to bind these receptors, for example, the pyridyl ethers (**ii**) [2] and the alkyne derivative **iii** [3]. Dogruer *et al.* [4] reported the synthesis of a series of analogues of **iii** and the subsequent investigation of their structure–affinity relationships for binding to nACh receptors. Because tertiary amines related to **i** and **ii** are generally better ligands than their corresponding secondary amines [5], the terminal amine substituents of **iii** were studied. In addition, the influence of a substituent at the 6-position of the pyridine ring and the replacement of the nitrogen atom of the pyridine ring by CH were examined. The novel compounds (**iiia-f, iv**) were tested in binding studies with nACh receptors using [^{3}H]-nicotine as a radioligand. Compounds **iiia** and **iiib** showed K_i values of 510 nM and 1.77 μM, respectively; in the same experiment **iii** had K_i of 113 nM. The pyrrolidinyl analogue (**iiic**) lacked affinity, whereas the *N,N,N*-trimethyl quaternary amine **iiid** had a K_i of 470 nM. Substitution of a chlorine atom at the 6-position of the pyridine ring to generate **iiie** (K_i of 154 nM) had little impact on the potency of the compound with respect to the potency of **iii**. In contrast, the 6-OCH_3 substituted (**iiif**) and the de-aza analogue of **iii** (**iv**) were inactive. These data indicate that the influence of substitution at the 6-position on affinity is analogous to the effect reported for **i** and **ii** [6]. However, the amine substituents seem to influence these ligands differently.



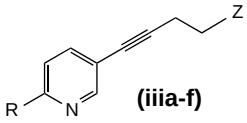
(i)



(ii)

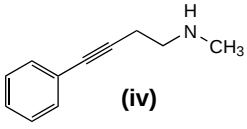


(iii)



(iiia-f)

(iiia) R = H, Z = $N(CH_3)_2$
(iiib) R = H, Z = $N(CH_3)C_2H_5$
(iiic) R = H, Z = N
(iiid) R = H, Z = $N(CH_3)_3^+$
(iiie) R = Cl, Z = $NHCH_3$
(iiif) R = OCH_3, Z = $NHCH_3$



(iv)

These results led the authors to conclude that the series **iii** compounds do not bind in a manner that parallels the binding of **ii**. The authors suggest that compound **ii** might bind using the same pyridine group, but different amine sites. Alternatively, if they use a common amine site, the amine substituents should be oriented differently.

Finally, it has been suggested that compound **iii** does not conform to the model proposed by Sheridan *et al*. [7], although it seems to conform to the vector-based pharmacophore model proposed by Olesen and co-workers [8].

1 Arneric, S.P. *et al.* eds. (1999) *Neuronal Nicotinic Receptors*, Wiley-Liss
2 Ferretti, G. *et al.* (2002) Homoazanicotine: a structure–affinity study for nicotinic acetylcholine (nACh) receptor binding. *J. Med. Chem.* 45, 4724–4731
3 Crooks, P.A. *et al.* (1997) US Patent 5,616,707

4 Dogruer, D. *et al.* (2004) 3-(4-Aminobutyn -1-yl)pyridines: binding at $\alpha_4\beta_2$ nicotinic cholinergic receptors. *Bioorg. Med. Chem. Lett.* 14, 523–526
5 Glennon, R.A. *et al.* (2000) Central nicotinic receptor ligands and pharmacophores. *Pharm. Acta Helv.* 74, 103–114
6 Lee, M. *et al.* (2002) A comparison of the binding of three series of nicotinic ligands. *Bioorg. Med. Chem. Lett.* 12, 1989–1992
7 Sheridan, R.P. *et al.* (1986) The ensemble approach to distance geometry: application to the nicotinic pharmacophore. *J. Med. Chem.* 29, 899–906
8 Tønder, J.E. *et al.* (2001) Agonists at the $\alpha_4\beta_2$ nicotinic acetylcholine receptors: structure–activity relationships and molecular modeling. *Curr. Med. Chem.* 8, 651–674

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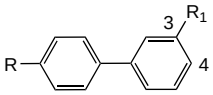
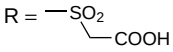
Small molecule inhibitors of the E1 helicase of human papillomavirus

Papillomaviruses are small DNA viruses that infect and replicate in the cutaneous or mucosal epithelia of human and other mammals. These viruses share a common genomic organization. However, the only encoded protein that has enzymatic activity is E1, a DNA helicase. High-throughput screening of a collection of Boehringer Ingelheim compounds was performed to measure the ATPase activity of recombinant human papillomavirus 6 (HPV6, the virus responsible for the majority of cases of genital warts) E1 helicase. The screening revealed **va** as a lead compound (IC_{50} of 2 μM), which exhibited the characteristics of a specific, reversible inhibitor able to interfere with the affinity of E1 helicase for ATP. Modifications of the lead compound showed that, although the sulfonylacetic acid moiety was not tolerant to modification, substitution at the 3 and 4 positions was possible. Thus, a parallel synthesis approach afforded several highly active compounds (e.g. **vb**, IC_{50} of 0.004 μM). However, these compounds were not active in a cell-based assay of HPV replication, probably as a result of the high polarity of the negatively charged sulfonylacetic acid group or the chemical instability of this group. The corresponding nitromethyl sulfone **vc**, (IC_{50} of 0.63 μM) was shown to be permeable in a cellular assay (Caco-2) and, even if it is not potent enough to show cell-based activity, can be considered a new lead compound [9].

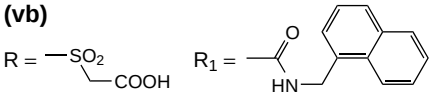


(v)

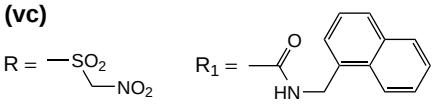
(va)



(vb)



(vc)



9 Faucher, A-M. *et al.* (2004) Discovery of small molecule inhibitors of the ATPase activity of Human Papillomavirus E1 Helicase. *J. Med. Chem.* 47, 18–21

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Biology

Microbiology

S. pyogenes vascular leakage can be inhibited by integrin antagonist

Streptococcus pyogenes can cause severe tissue damaging and systemic infections with high mortality. Hallmarks of systemic disease (toxic shock syndrome, STSS) are severe hypotension and multi-organ failure.

One of the most studied virulence factors of *S. pyogenes* is the cell wall-anchored M protein, which confers resistance against bacterial killing by neutrophils and binds several human proteins, including fibrinogen. M protein is initially anchored to the cell wall of the bacterium but can be released by proteinases, however, the pathophysiological consequences of this have not been investigated in detail.

Herwald *et al.* [1] show that proteinases secreted from human stimulated neutrophils release several fibrinogen-binding M protein fragments, which in turn trigger the neutrophils to aggregate and release the inflammatory mediator heparin-binding protein (HBP). The aggregation and activation of neutrophils was not directly mediated by M protein, but rather M protein–fibrinogen complexes.

Experiments using a β2 integrin peptide antagonist (Gly-Pro-Arg-Pro) showed that M protein–fibrinogen complexes activate neutrophils by β2 integrin crosslinking. Furthermore, intravenous injection of M protein into mice caused severe hemorrhagic lung lesions that could be reversed by co-injection of Gly-Pro-Arg-Pro. Finally, analysis of infected soft tissue from a patient with necrotizing fasciitis and STSS revealed that M protein is released from bacteria into the surrounding tissue and is co-localized with fibrinogen.

Overall, this study elegantly elucidates a series of events that might explain the rapid progression from localized *S. pyogenes* infection to severe systemic disease. In addition, the results obtained with the β2-integrin peptide antagonist could represent a much needed novel therapeutic strategy against STSS.

1 Herwald, H. (2004) M protein, a classical bacterial virulence determinant, forms complexes with fibrinogen that induce vascular leakage. *Cell* 116, 367–379

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