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.**

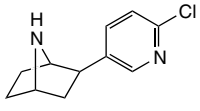
Chemistry

Molecules

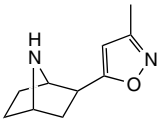
8-Azabicyclo[3.2.1]octane derivatives as epibatidine analogues

The amphibian alkaloid (-)-epibatidine (**i**) [1] is a highly potent nicotinic acetylcholine receptor (nAChR) agonist at neuronal receptor subtypes ($\alpha4\beta2$ and $\alpha7$,) as well as at nAChRs in peripheral autonomic ganglia and skeletal muscle [2,3]. However, its therapeutic potential is limited due to its acute toxicity at doses only slightly higher than its effective analgesic dose [4–6].

Substitution of the chloropyridyl ring of compound **i** with an isoxazole ring led to epiboxidine (**ii**), which, although less potent than **i**, showed a better activity:toxicity ratio [7]. As a continuation of their studies [8,9] on the 8-azabicyclo[3.2.1] moiety aimed to identify less toxic analogues of epibatidine, Trudell and collaborators have recently reported their results on a series of 2- and 3-isoxazolyl-8-azabicyclo[3.2.1]octanes



(i)



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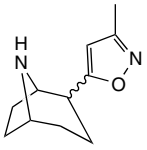
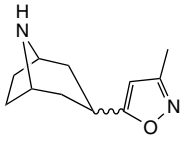


(iii a,b)



(iv a,b)

[10]. All compounds were tested for their ability to inhibit [^{3}H] cytisine binding in homogenates of rat striatum, where the $\alpha4\beta2$-subtype is the predominant nAChR [11].

As expected, all compounds showed lower binding affinity with respect to epibatidine. It should be noted that both the positional attachment and the stereochemical orientation of the isoxazolyl group had a dramatic effect on binding affinities. In particular, the β-isomers were at least two orders of magnitude more potent than the corresponding α-isomers. In addition, substitution at the 2-position (compounds **iiia,b**) was significantly favoured over the 3-position (compounds **iva,b**).

The most potent compound was 2β-isoxazolyl-8-azabicyclo[3.2.1]octane (**iiia**, *K*i = 3 nM). The 3-β isomer (**iva**) was 50-fold less potent. In the same experiment, epibatidine and nicotine showed *K*i values of 0.79 nM and 8.0 nM, respectively. It should also be noted that, consistent with the SAR studies of epibatidine and epiboxidine, compound **iiia** was slightly more potent than its corresponding *N*-methyl analogue. Further studies are currently in progress to verify whether, as for epiboxidine, compound **iiia** has an improved therapeutic ratio relative to epibatidine.

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Biology

Microbiology

From ubiquity to specificity in drug target

18 million people in the world are suffering from the protozoan infection, Chagas' disease and, in the absence of vaccines and cures, there is an urgent need to identify protein targets for the development of therapeutic agents. Essential proteins that are ubiquitous are often neglected as targets for drug design. However, resolution of the fine structure can show some specificity that could be used to avoid cross-reactivity in drug targeting. Harkiolaki *et al.* now report the crystal structure of the dUTPase from *Trypanosoma cruzi* in its native state and complexed to dUDP [1]. By comparison of structures, the authors concluded that this ubiquitous and essential protein could represent a potential target in the treatment of Chagas' disease.

The major observation was that the dUTPase of *T. Cruzi*, which is a dimer, presents no structural similarities with the other known dUTPases (which are trimeric for the most part). Hence, it represents a novel protein fold. Additionally, despite a similar function at comparable kinetics, the dUTPase from *T. cruzi* could present a catalytic mechanism that is quite different from the other dUTPases.

Intriguingly, the substrate is bound to the active site in a different manner than for the other dUTPases and when the authors superimposed the nucleotide models derived from complexes of trimeric dUTPases and dimeric *T.cruzi* dUTPases, they observed a different nucleotide conformation. Specifically, on the uracil moiety, the deoxyribose of dUDP in the *T.cruzi* complex deviates significantly from all other deoxyriboses. Similarly, when the superimposition is performed on the deoxyribose and α-phosphate atoms, the plane of the uracil moieties of dUDP in the *T.cruzi* enzyme is rotated relative to the planes of the uracil moieties of nucleotides in complex with trimeric dUTPases.

Theses differences could be exploited in drug design for the production of a nucleotide mimic to bind and selectively inactivate the dimeric dUTPase. Moreover, as in *T.cruzi,* this enzyme also exists in *Trypanosoma brucei* and *Leishmania major* and no trimeric dUTPase homolog has been identified in their genomes. Consequently, this protein could be considered as a good potential target for drug design against protozoan infections.

1 Harkiolaki, M. *et al.* (2004) The crystal structure of *Trypanosoma cruzi* dUTPase reveals a novel dUTP/dUDP binding fold. *Structure* 12, 41–53

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Staphylococcal protein A induces inflammation in the airways

Immunoglobulin-binding surface proteins are widespread among Gram-positive pathogens and are considered important for pathogenesis. The prototypical protein A from *Staphylococcus aureus* interferes with opsonization by binding the Fc portion of immunoglobulins. *S. aureus* isolated from airway infections show increased expression of protein A, suggesting a role for this protein in interactions with airway epithelium in the absence of immunoglobulins. In staphylococcal pneumonia, neutrophils (PMNs) infiltrate to control the bacteria, but also cause epithelial damage and impairment of lung function.

Gómez *et al.* [2] studied the interactions between purified protein A, protein A-expressing and non-expressing *S. aureus* strains with airway epithelial cells. Protein A or protein A-overexpressing strains, but not non-expressing strains, induced interleukin 8 (IL-8) through mitogen-activated kinases (MAPKs). By using a protein A magnetic bead screening assay of cell lysates, TNFR1, the receptor for tumor necrosis factor-α (TNF-α), was identified as the protein A receptor. Soluble protein A or S. aureus stimulated surface expression and shedding of TNFR1 and induced inflammation by mimicking TNF-α activation through TNFR1. A model of pneumonia in mice showed that lack of either protein A in bacteria or TNFR1 in mice resulted in decreased virulence, and administration of soluble TNFR1 blocked protein A-induced inflammation, including PMN recruitment.

In summary, this study identifies an important new interaction between staphylococcal protein A and a receptor (TNFR1) in airway epithelium that directly stimulates inflammatory responses with neutrophil recruitment known to contribute to the pathology seen in pneumonia. This information provides opportunities for development of therapeutic strategies, and it is encouraging that the soluble form of the receptor functions as an inhibitor of the inflammation induced by staphylococci.

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Cancer biology

Alternatives to Herceptin

The monoclonal antibody Herceptin (Trastuzumab) has become an important