Monitor

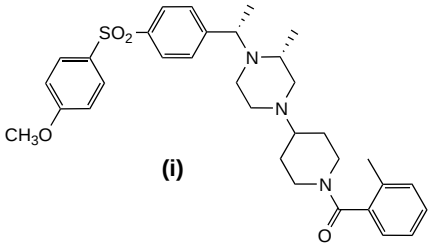
Monitor provides an insight into the latest developments in the pharmaceutical and biotechnology industries. ***Chemistry*** examines and summarises recent presentations and publications in medicinal chemistry in the form of expert overviews of their biological and chemical significance, while ***Profiles*** 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

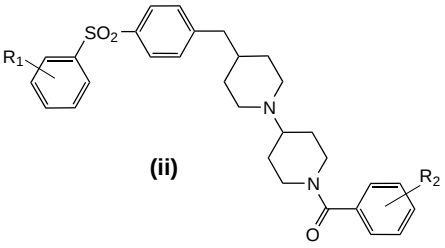
Molecules

Muscarinic M_2 acetylcholine receptor antagonists

Alzheimer's disease (AD) is characterized by a progressive loss of cognitive functions and has been associated with the impairment of the cholinergic system. Currently, therapeutic intervention in AD involves the enhancement of the levels of the neurotransmitter acetylcholine (ACh) through the administration of acetylcholinesterase inhibitors. An alternative approach involves the development of drugs that enhance the release of ACh through antagonism of central presynaptic muscarinic M_2 acetylcholine receptors. However, it is important to obtain selective binding to M_2 receptors relative to the other muscarinic receptor subtypes, because the postsynaptic M_1 receptors are believed to mediate the effects of ACh in learning and memory and antagonism of these receptors produces cognitive deficits. Furthermore, the peripheral M_3 receptors are involved in the regulation of gastrointestinal functions. Recently, the piperazine derivative **i** was shown to be a selective M_2 high-affinity ligand (K_i 2.6 nM; selectivity of **i** for the M_2 receptor versus the other muscarinic receptor subtypes is expressed as the K_i ratio: M_1:M_2 of 245; M_3:M_2 of 118) [1]. However, the overall pharmacokinetic profile of this class of M_2 antagonists was poor, resulting in low plasma levels after oral administration and

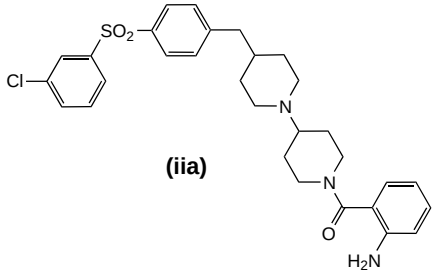


(i)



(ii)

low efficacy in several animal models. From the structure–activity relationships obtained for **i** [1], the achiral core of **ii** was selected for modification [2]. From the derivatives synthesized, the anthranilamides, and in particular **iia**, showed good *in vitro* activity (K_i 0.89 nM for M_2 receptors; K_i ratios for **iia** selectivity: M_1:M_2 of 734; M_3:M_2 of 787; M_4:M_2 of 69; M_5:M_2 of 95). In *in vivo* assays, **iia** caused a significant increase in ACh release (rat microdialysis assays) and was active in animal models of cognition with a maximally effective oral dose of 0.1 mg kg^{-1} [2]. Based on these results, **iia** was selected for clinical evaluation as a potential new treatment for AD.



(iia)

1 McCombie, S.W. *et al.* (2002) Synthesis and structure–activity relationships of M_2-selective muscarinic receptor ligands in the 1-[4-(4-arylsulfonyl)-phenylmethyl]-4-(4-piperidinyl)-piperazine family. *Bioorg. Med. Chem. Lett.* 12, 795–798
2 Clader, J.W. *et al.* (2004) Muscarinic M_2 antagonists: anthranilamide derivatives with exceptional selectivity and *in vivo* activity. *Bioorg. Med. Chem.* 12, 319–326

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Combinatorial chemistry

Trypsin and chymotrypsin inhibitors

The trypsin inhibitors CMTI-I and CMTI-III (*Cucurbita maxima* trypsin inhibitor) are the first two members of a family that are the lowest molecular mass serine proteinase inhibitors isolated to date. Despite their small size, CMTI are potent trypsin inhibitors with association equilibrium constants (K_a) for CMTI-I and CMTI-III of 3.2×10^{11} and 6.8×10^{11} M^{-1}, respectively, against bovine β-trypsin [3]. The peptides

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contain a reactive peptide bond (P_1–P_1') located between the Arg5 and Ile6 residues. X-ray crystallography has demonstrated that 13 amino acid residues (residues 1–7, Glu9 and residues 25–29) of CMTI-I are directly involved in its interaction with trypsin. These inhibitors potentially have therapeutic applicability in the treatment of cystic fibrosis and pancreatic disease, as well as in antiretroviral therapy approaches. Recently, Kazmierczak *et al*. [4] investigated the *N*-terminal segment of CMTI, which is directly involved in the interaction with bovine β-trypsin, as a possible template to design low-molecular-mass proteinase inhibitors. A solid-phase library of 2016 decapeptides in mixtures was synthesized on TentaGel S Ac with attached Fmoc-Gly resin (RAPP Polymere; http://www.rapp-polymere.com). Deconvolution identified active peptide constituents. The decapeptides were screened against bovine β-trypsin and bovine α-chymotrypsin. One of the most potent deconvoluted compounds isolated was **iii,** which possessed a K_a of 1.1 x 10^8 M^{-1} against bovine β-trypsin. Although this analogue is three- to fourfold less active than wild-type CMTI inhibitors, this type of analogue has a lower molecular weight and fewer conformational constraints than wild-type inhibitors. Thus, these decapeptides represent promising starting points for the optimization of these low-molecular-weight serine proteinase inhibitors and this work warrants further investigation.

[Homoarginine-Val-Gly-Pro-Arg-Ile-Leu-Met-Homoarginine-Gly]CMTI-$III_{1–10}$

(iii)

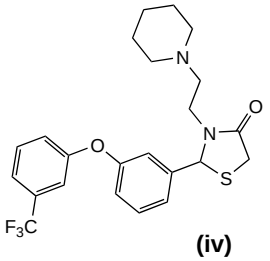
3 Wieczorek, M. *et al*. (1985) The squash family of serine proteinase inhibitors. Amino acid sequences and association equilibrium constants of inhibitors from squash, zucchini, and cucumber seeds. *Biochem. Biophys. Res. Commun.* 2, 646–652

4 Kazmierczak, K. *et al*. (2003) Selection of low-molecular-mass trypsin and chymotrypsin inhibitors based on the binding loop of CMTI-III using combinatorial chemistry methods. *Biochem. Biophys. Res. Commun.* 310, 811–815

Sodium channel agonists

Chronic pain in humans represents a disease for which there is currently a significant unmet medical need. According to statistics from the American Society of Anesthesiologists (http://www.asahq.org), back pain disables five million people per year in the US. Another 66 million people experience pain related to arthritis, 40 million people have recurrent severe headaches and 90% of terminal cancer patients suffer from severe pain associated with the disease. Prescribed non-steroidal anti-inflammatory agents, over-the-counter pain medication or, in the most severe cases, opioids are the front-line therapies currently used to treat the condition. Most of these treatments have undesirable side effects associated with their application. The discovery of a broad spectrum, highly effective analgesic agent that is also devoid of side effects is a common goal within the pharmaceutical industry.

Ion channels that regulate the excitability of sensory neurons might represent plausible drug targets for the treatment of chronic pain. Specifically, voltage-gated sodium channels are attractive targets because they are essential for the inhibition and propagation of neuronal impulses. Furthermore, they play a crucial role in determining the activation threshold and firing patterns in injured primary afferent neurons. Thus, optimization of sodium channel antagonist pharmacology as an approach to the discovery of new analgesic agents has been a recent goal for Sun *et al*. [5]. A library of 91 thiazolidinone compounds was synthesized as singletons in solution. For the measurement of K_i values of synthesized compounds, oocytes were held at a holding potential of –120 mV followed by a four second depolarizing step ('pre-pulse') to –10 mV in which the maximum current was elicited. At the end of this depolarization step, nearly all of the channels would be in the inactivated state. Next, a 10 ms hyperpolarizing step was performed to remove some channels from the inactivated state. A final depolarizing step ('test pulse') was used to assay the sodium current following this prolonged depolarization. Sodium currents were measured at this test pulse before and after drug application. One of the most potent compounds found was **iv,** which displayed a K_i of 90 nM. This work has produced potent and novel sodium channel antagonists that have improved pharmaceutical and pharmacological properties and further work in this area is warranted.



(iv)

5 Sun, Q. *et al*. (2003) Parallel synthesis of a biased library of thiazolidinones as novel sodium channel antagonists. *Comb. Chem. High Through. Screen.* 6, 481–488

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Biology

Microbiology

Streptococcus pyogenes IL-8 protease is inhibited by pheromone

The strictly human pathogen *Streptococcus pyogenes* causes relatively mild localized upper respiratory tract and skin infections, but occasionally causes severe invasive and/or tissue-destroying infections with high mortality. *S. pyogenes* has evolved several strategies to evade the immune response by binding to plasma proteins and immune regulators, as well as immuno-modulating enzymes interacting with the immune system [1].

Hidalgo-Grass *et al*. [2] analyzed *S. pyogenes* isolates and tissues from patients with necrotizing infections. Infected tissue contained numerous bacteria, but no infiltrating neutrophils. Bacteria were shown to release a protease activity that degrades the human chemokine interleukin 8 (IL-8), which is important for neutrophil recruitment. In a mouse model of soft-tissue infection the bacteria produced fatal necrotic infections with reduced neutrophil infiltration. Furthermore, the strains were mutated in *silCR*, encoding the pheromone peptide

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