DDT Vol. 6, No. 5 March 2001

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.

Monitor Editor: Debbie Tranter

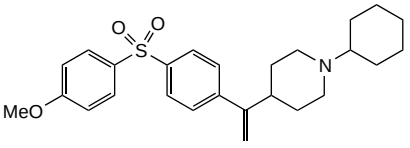
Monitor Contributors:
David Barrett, *Fujisawa Pharmaceutical Company*
Steven Langston, *Millennium Pharmaceuticals*
Paul Edwards, *Pfizer*
Michael Walker, *Bristol-Myers Squibb*
Andrew Westwell, *Nottingham University*
John Weidner, *Emisphere*
Daniela Barlocco, *University of Milan*

Molecules

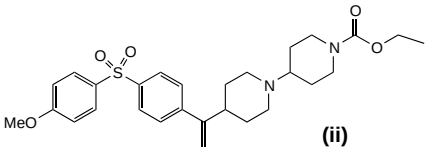
Potent M_2 muscarinic receptor antagonists

Methods that increase the level of acetylcholine (ACh), a key component in learning and memory, represent a strategy for the treatment of Alzheimer's disease. ACh levels can be increased by inhibition of acetylcholinesterase, but can also be potentially increased by inhibition of presynaptic muscarinic M_2 receptors or activation of post-synaptic M_1 receptors. Efforts to discover potent M_2 receptor antagonists, therefore, require good selectivity relative to M_1 receptors and also to the related M_3 receptors, inhibition of which could lead to undesirable effects. Recently, workers at Schering-Plough (Kenilworth, NJ, USA) have described several related compounds that display M_2-selective antagonist activity and enhancement of brain ACh release after oral administration in rats[1,2].

Compound **(i)** was identified as a lead compound with potent M_2 antagonist activity (K_i = 0.17 nM), but poor selectivity towards other subtypes (M_1: K_i = 2.8 nM; M_1/M_2: K_i = 16 nM; M_3: K_i = 0.48 nM; M_3/M_2: K_i = 3 nM)[1]. A design strategy based on extending the core structure of **(i)** to generate new potential binding sites was investigated. Compound **(ii)** was identified as a potent M_2 antagonist (K_i = 0.11 nM) with improved selectivity over the other subtypes (M_1/M_2: K_i = 59 nM; M_3/M_2: K_i = 34 nM), and also significantly enhanced ACh levels after oral administration at 10 mg kg^{-1}, suggesting good blood–brain barrier penetration.

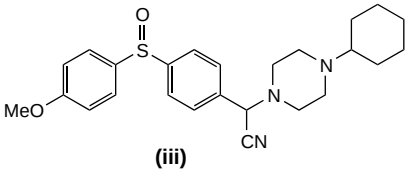


(i)



(ii)

In a related study by the same group[2], compound **(iii)** was identified as an M_2-selective antagonist by optimization of the screening lead [4-(phenylsulfonyl) phenyl]methylpiperazine. Sulfoxide **(iii)** demonstrated a good affinity for M_2 receptors (K_i = 2.7 nM) and good subtype



(iii)

selectivity (M_1/M_2 = 40). The cyano group was shown to undergo racemization readily, thus preventing assay of the individual enantiomers. However, compound **(iii)** was shown to produce a dose-dependent increase in ACh levels in rat brain after oral administration, and to be effective in animal models of cognition.

1 Wang, Y. *et al.* (2000) Design and synthesis of piperidinyl piperidine analogues as potent and selective M_2 muscarinic receptor antagonists. *Bioorg. Med. Chem. Lett.* 10, 2247–2250

2 Kozlowski, J.A. *et al.* (2000) Diphenyl sulfoxides as selective antagonists of the muscarinic M_2 receptor. *Bioorg. Med. Chem. Lett.* 10, 2255–2257

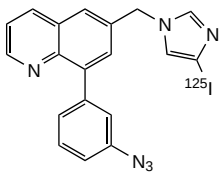
A potent photoaffinity probe inhibitor of PDE4

The development of inhibitors of type-4 cAMP-specific phosphodiesterase (PDE4) offers potential for new treatments for asthma. PDE4 hydrolyzes the second messenger cAMP to AMP in a variety of cells, and inhibitors of this enzyme lead to increased concentrations of cAMP. Various proinflammatory processes, and bronchospasm, have been shown to be ameliorated after administration with PDE4 inhibitors, leading to a widespread interest in compounds with this mechanism of action. One of the principal problems with PDE4 inhibitors, to date, has been the observation of emesis as a side effect. Therefore, the ultimate goal would be to identify the target sites responsible for manifestation of both the desirable PDE4 inhibition effect and the undesirable emetic effect. To this end,

 PII: S1359-6446(00)01674-3

researchers at Merck Frosst (Kirkland, Quebec, Canada) recently described the identification of compound **(iv)** as an efficacious PDE4 inhibitor and an emetic that could be used as a photoaffinity probe for the respective biological target sites[3].

Using several known PDE4 inhibitors as starting materials, functional groups suitable for photoaffinity labelling were attached to various positions, and compounds that retained emetic and PDE4 inhibitory effects were screened.



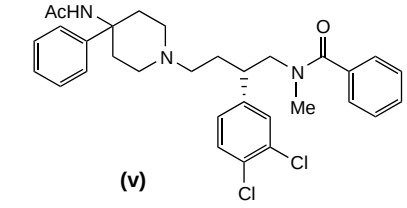
(iv)

Compound **(iv)** was identified as a potent inhibitor of PDE4 (IC_{50} = 0.4 nM) and also caused emesis at a low oral dose (0.1 mg kg^{-1}) in the ferret. Additionally, compound **(iv)** was shown to label recombinant PDE4 in the presence of other cellular proteins under typical photolysis conditions. Competition experiments with the known active-site-directed PDE4 inhibitor CDP840 established that **(iv)** is an active-site-directed photoprobe. This compound could help to clarify the respective efficacy and emesis target sites and, ultimately, should assist in the identification of more potent, non-emetic inhibitors of PDE4.

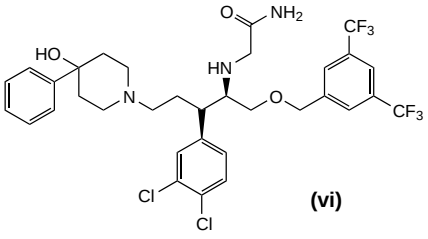
3 Macdonald, D. *et al.* (2000) Hunting the emesis and efficacy targets of PDE4 inhibitors: identification of the photoaffinity probe 8-(3-azidophenyl)-6-[(4-iodo-1*H*-1-imidazolyl)methyl]quinoline (APIIMQ). *J. Med. Chem.* 43, 3820–3823

Novel dual antagonists of neurokinin receptors 1 and 2

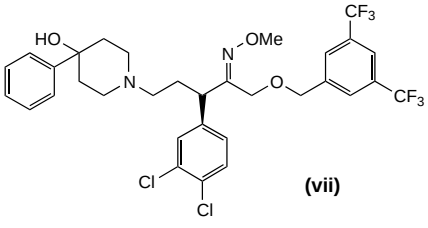
Dual inhibitors of neurokinin 1 (NK_1) and neurokinin 2 (NK_2) receptors could be beneficial in the treatment of asthma, by the simultaneous antagonism of the effects of substance P and neurokinin A. Using a known NK_2 antagonist as a scaffold for chemical modification, researchers at Schering-Plough (Kenilworth, NJ, USA) have recently described the design and synthesis of a novel series of dual NK_1/NK_2 antagonists[4,5]. Using the NK_2-selective compound SR48968 **(v)** as a lead, exploration of sites compatible with maintaining the potent NK_2 inhibitory activity (NK_2: K_i = 0.5 nM) were first identified, followed by the introduction of an NK_1 pharmacophore to the basic scaffold. Compounds **(vi)** and **(vii)** were identified as novel dual NK_1 and NK_2 antagonists [**(vi)**: NK_1, K_i = 9 nM, NK_2, K_i = 34 nM; **(vii)**: NK_1, K_i = 20 nM, NK_2, K_i = 7 nM], by a receptor binding assay using membrane preparations from Chinese hamster ovary cells containing recombinant NK_1 and NK_2 receptors.



(v)



(vi)



(vii)

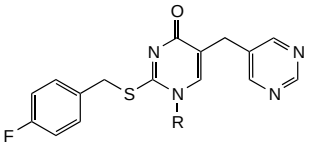
4 Reichard, G.A. *et al.* (2000) The design and synthesis of novel NK1/NK2 dual antagonists. *Bioorg. Med. Chem. Lett.* 10, 2329–2332

5 Ting, P.C. *et al.* (2000) Synthesis and NK_1/NK_2 receptor activity of substituted-4-(*Z*)-(methoxyimino) pentyl-1-piperazines. *Bioorg. Med. Chem. Lett.* 10, 2333–2335

Inhibitors of lipoprotein-associated phospholipase A_2

Atherosclerosis is a highly complex biochemical process that ultimately manifests itself in the form of many significant medical conditions. The reduction of serum cholesterol levels is known to be a successful treatment for atherosclerotic disease; however, new approaches that can potentially effect the production or action of the atherogenic low-density lipoprotein (LDL) could offer hope for improved clinical outcomes. LDL is thought to be involved in the development of atherosclerosis by its oxidative modification. Furthermore, a phospholipase associated with LDL is involved in the hydrolysis of oxidized LDL, a process that results in the release of lysophosphatidylcholine and oxidized fatty acids. Inhibitors of this lipoprotein-associated phospholipase (Lp-PLA_2) are of interest as potential new treatments for atherosclerosis. Recently, researchers at SmithKline Beecham (Harlow, UK) have described a series of potent nanomolar inhibitors of this enzyme that also display oral activity[6].

Earlier reports described various pyrimidines and natural product derivatives as inhibitors of Lp-PLA_2, although these had poor *in vivo* activity because of low plasma levels[7,8]. Further optimization studies led to the identification of compounds **(viii)** and **(ix)**, with amide moieties incorporated into novel *N*1-substitutents on the pyrimidine scaffold,



(viii) R = (*E*) $(CH_2)_3CONH(CH_2)_8CH{=}CHC_8H_{17}$
(ix) R = (*Z*) $(CH_2)_3CONH(CH_2)_8CH{=}CHC_8H_{17}$

which were much more potent in whole plasma. In particular, compound **(ix)** caused 85% inhibition of plasma Lp-PLA_2 in Watanbe hereditable hyperlipidaemic (WHHL) rabbits at an oral dose of 10 mg kg^{-1}, and potent *in vitro* inhibition

in the absence of plasma, with an IC_{50} value of 0.4 nM. This compound also had a prolonged duration of action, suggesting potential for further evaluation of the role of Lp-PLA_2 inhibitors in atherosclerotic disease.

6 Boyd, H.F. *et al.* (2000) *N*-1 substituted pyrimidin-4-ones: novel, orally active inhibitors of lipoprotein-associated phospholipase A_2. *Bioorg. Med. Chem. Lett.* 10, 2557–2561

7 Boyd, H.F. *et al.* (2000) 2-(Alkylthio)pyrimidin-4-ones as novel, reversible inhibitors of lipoprotein-associated phospholipase A_2. *Bioorg. Med. Chem. Lett.* 10, 395–398

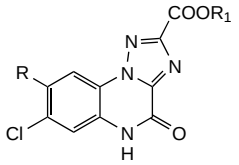
8 Pinto, I.V. *et al.* (2000) Natural product derived inhibitors of lipoprotein associated phospholipase A_2, synthesis and activity of analogues of SB253514. *Bioorg. Med. Chem. Lett.* 10, 2015–2017

Heterocyclic-fused quinoxalinones as selective antagonists at the AMPA receptor

Glutamate (Glu) is a potent excitatory transmitter in the CNS, and might also be involved in many pathological processes. The excitotoxicity of Glu is mainly mediated by the overstimulation of the ionotropic Glu receptors (iGluRs), namely: *N*-methyl-D-aspartate (NMDA), 2-(aminomethyl)phenylacetic acid (AMPA) and kainic acid (KA) receptors.

Several 4,5-dihydro-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates (TQXs) were previously reported to have significant affinity for both the glycine/NMDA and AMPA receptors[9]. The same research group has recently extended its studies on this tricyclic system, with the aim of obtaining more selective antagonists for these receptors[10]. In particular, because the presence of an imidazol-1-yl substituent **(x)** on the triazoloquinoxaline moiety was claimed to be essential for high AMPA receptor activity[11], the researchers introduced this heterocycle instead of the 8-NO_2 group present in the unselective model **(xi)**.

The 1,2,4-triazol-4-yl substituted molecule was also studied **(xii)**. The binding results demonstrated that the presence at the 8-position of a nitrogen-containing heterocycle confers selectivity for the AMPA receptor to this series; the triazolyl derivatives **(xii)** were the most potent compounds. As previously seen in the model **(xi)**, the presence of a free carboxylic acid at position 2 was not essential to the selectivity for the AMPA receptor, because the corresponding esters were found to be almost equipotent.



(x) R = imidazol-1-yl
(xi) R = NO_2
(xii) R = 1,2,4-triazol-4-yl

(x)–**(xii)**, R_1 = H, Et

The compounds were also tested for their functional antagonistic activity. The results of the electrophysiological assays closely correlate with the binding data on AMPA and glycine/NMDA receptors. Compound **(xii)**, as the free acid, was the most potent and selective. Indeed, its inhibitory activity on depolarization induced by 5 μM AMPA was much higher than that on NMDA-evoked responses (IC_{50} = 2.3 ± 0.4 μM and 46 ± 4 μM, respectively).

These results indicate that in the quinoxaline derivatives the triazolyl moiety is an effective substitute for the imidazolyl ring for providing potent and selective AMPA antagonists.

9 Catarzi, D. *et al.* (1999) 4,5-Dihydro-1,2,4-triazolo[1,5-*a*]quinoxalin-4-ones: excitatory amino acid antagonists with combined glycine/NMDA and AMPA receptor affinity. *J. Med. Chem.* 42, 2478–2484

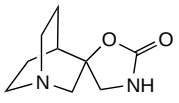
10 Catarzi, D. *et al.* (2000) 7-Chloro-4,5-dihydro-8-(1,2,4-triazol-4-yl)-4-oxo-1,2,4-triazolo[1,5-*a*]quinoxaline-2-carboxylates as novel highly selective AMPA receptor antagonists. *J. Med. Chem.* 43, 3824–3826

11 Chimirri, A. *et al.* (1999) AMPA receptor antagonists. *Exp. Opin. Ther. Patents* 9, 557–570

The spirooxazolidinone moiety as a potential substrate for selective agonists at the α7 nicotinic acetylcholine receptor

Neuronal nicotinic acetylcholine receptors (nAChRs) are cation channels comprising five subunits; they can be either homo-oligomeric (α subunits) or hetero-oligomeric (α and β subunits). Their role in modulating neurotransmission has recently been demonstrated by several studies. This has led to a renewed interest in the identification of nicotinic agonists for the treatment of neurodegenerative disorders. Because our knowledge of the subtypes that mediate the central action of nicotine is limited, the α7 and α4β2, which are largely distributed in the CNS, have been indicated as potential targets. Although there are several nicotinic agonists selective for the α4β2 subtype, only anabaseine analogues were reported to be functionally selective for the α7 subtype[12].

Researchers from AstraZeneca (Alderley Edge, UK) have recently reported their preliminary results on several spirooxazolidinone derivatives as potential agonists at the α7 nicotinic receptor[13]. In particular, the (–)-spiro-(1-azabicyclo[2.2.2]octane-3,5′oxazolidin-2′-one) [(–)-**(xiii)**] was a potent full agonist (96% affinity) of the receptor, and the most selective α7 receptor agonist known thus far. The compound is fivefold more potent and 35,000-fold more selective than (–)-nicotine at this receptor. It should be noted that the (+)-enantiomer [(+)-**(xiii)**] displayed poor affinity and intrinsic activity for the same receptor. These results suggest that the location of the carbonyl functionality is crucial to mimicking acetylcholine in the α7 receptor. Data on slightly modified analogues of [(–)-**(xiii)**] (e.g. carbonate, ester or amide instead of the carbamate function) demonstrated a dramatic loss in affinity for the α7 receptor. Moreover, the insertion of a small alkyl group at the amide nitrogen of [(–)-**(xiii)**] significantly reduced its affinity for the α7 receptor.



(xiii)

These results strengthen the hypothesis that both the electronic nature and the location of the carbonyl group play an essential role in the functionality of this series as acetylcholine receptor agonists. The interesting profile of [(–)-**(xiii)**] makes this compound an important tool for the understanding of α7 nicotinic receptor function.

12 De Fiebre, C.M. *et al.* (1995) Characterization of a series of anabaseine-derived compounds reveals that 3-(4)-dimethylamino-cinnamylidene derivative is a selective agonist at neuronal nicotinic α7/^{125}I-α-bungarotoxin receptor subtypes. *Mol. Pharmacol.* 47, 164–171

13 Mullen, G. *et al.* (2000) (–)-spiro-[1-azabicyclo[2.2.2]octane-3,5′oxazolidin-2′-one], a conformationally restricted analogue of acetylcholine, is a highly selective full agonist at the α7 nicotinic acetylcholine receptor. *J. Med. Chem.* 43, 4045–4050

David Barrett
Fujisawa Pharmaceutical Company
2-1-6 Kashima, Yodogawa-ku
Osaka 532-8514, Japan
tel: +81 6 6390 12856
fax: +81 6 6304 5435
e-mail: David_Barrett@po.fujisawa.co.jp

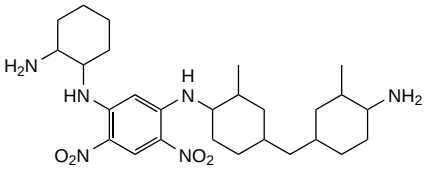
Daniela Barlocco
University of Milan
Viale Abruzzi, 42
Milano-20131, Italy
tel: +39 02 2950 2223
fax: +39 02 2951 4197
e-mail: daniela.barlocco@unimi.it

Combinatorial chemistry

Antibacterial compounds

Gram-positive bacteria have become increasingly resistant to antimicrobial agents, and hence multi-drug-resistant bacterial pathogens are now a major problem in clinical medicine. *Staphylococcus aureus* is a common human pathogen that has become increasingly difficult to treat because of its resistance to antimicrobial agents. Vancomycin is the main antimicrobial treatment for infections caused by *S. aureus* strains that cannot be treated with penicillinase-resistant antibiotics. The emergence of vancomycin-resistant *Enterococcus* species raises the threat of possible transfer of resistance factors to *S. aureus*. Vancomycin-resistant *Stapylococcus* clinical isolates have already been discovered in Japan. There is, therefore, a need for new antimicrobial agents.

A solution-phase approach was used to identify compounds with novel antibacterial activity[1]. A library of 4,900 compounds was prepared in mixtures of ten from a solution-phase sequential displacement of two fluorines on the 1,5-difluoro-2,4-dinitrobenzene core library template using a set of 70 amines. The mixtures of ten were tested for antibacterial activity against *S. aureus* and *E. faecalis* and several active mixtures were identified. All compounds contained within these mixtures were resynthesized as single compounds and re-tested. One of the most potent compounds isolated was **(i)**. This compound possessed a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *S. aureus* of 11.09 μg ml^{-1} and 22.18 μg ml^{-1}, respectively, and MIC and MBC values against *E. faecalis* of 5.5 μg ml^{-1} and 11.0 μg ml^{-1}, respectively. This work could, therefore, prove useful in the further optimization of the lead compounds identified in this library for the production of more potent novel antibacterial agents.



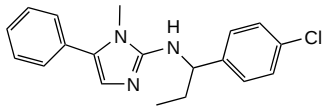
(i)

1 Lam, K.S. *et al.* (2000) Solution-phase synthesis of a 1,5-dialkylamino-2,4-dinitrobenzene library and the identification of novel antibacterial compounds from this library. *J. Comb. Chem.* 2, 467–474

Na^+/K^+ ATPase inhibitors

Congestive heart failure (CHF), a disease characterized by the failure of the heart to pump sufficient blood to meet the metabolic needs of the body, affects millions of people worldwide. The five-year mortality rate for CHF is almost 50%, and symptoms of the disease include peripheral oedema, tachycardia and decreased exercise capacity. Current treatments include diuretics, angiotensin-converting-enzyme inhibitors and digitoxin. Digitoxin has been shown to increase the exercise capacity of CHF patients via the inhibition of myocardial Na^+/K^+ ATPase, which strengthens the heart's pumping action (positive inotropy). Digitoxin plasma-concentrations, however, must be carefully maintained below the potentially life-threatening concentration of 2.0 ng ml^{-1}. Treatment with digitoxin is complicated further by high plasma-protein-binding and lipid solubility, which can extend the time and dosing required to achieve steady-state plasma concentrations (up to two weeks in some patients).

To develop positive inotropes with a more favourable therapeutic index, a library of aminoimidazoles was synthesized[2]. Thirteen individual compounds were synthesized in solution by condensation of a series of lithium amides with substituted heteroaryl sulfones. One of the most potent compounds isolated was **(ii),** which possessed an IC_{50} value of 800 nM against the isolated myocardial Na^+/K^+ ATPase enzyme. This work could be useful in the future for further defining the pharmacophore required for activity against myocardial Na^+/K^+ ATPase.



(ii)

2 Blass, B.E. *et al.* (2000) Parallel synthesis and evaluation of *N*-(1-phenylethyl)-5-phenylimidazole-2-amines as Na^+/K^+ ATPase inhibitors. *Bioorg. Med. Chem. Lett.* 10, 1543–1545

1359-6446/01/$ – see front matter PII: S1359-6446(00)01676-7