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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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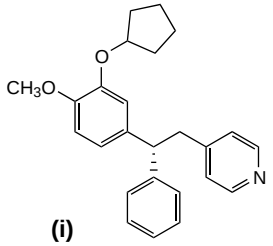
Molecules

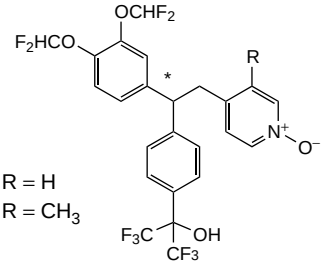
Improved pharmacokinetic parameters of PDE4 inhibitors

Phosphodiesterases comprise a large family of enzymes responsible for the breakdown of the cyclic nucleotide second messengers cAMP and cGMP. The phosphodiesterase isoform, PDE4, is highly expressed in inflammatory cells and airway smooth-muscle cells. An increase in cAMP levels is known to prevent the activation of such cells and, thus, the identification of selective inhibitors of PDE4 is an area of intense interest as potential anti-inflammatory agents and to alleviate bronchoconstriction, for example, in the treatment of asthma.

The potent PDE4 inhibitor CDP840 **(i)** has previously been reported by the Merck Frost Centre for Therapeutic Research (http://www.merck.com) and shown to reduce bronchoconstriction in animal models and asthmatic patients; however, it has a short half-life *in vivo*. The stabilization of CDP840 to metabolism has been reported by the group through replacing the alkoxy groups



(i)



(ii) R = H
(iii) R = CH_3

with difluoromethylene, and oxidation of the pyridine to the N-oxide to give molecule **ii** (R = H) [1]. This compound – L791,943 – exhibited a good biological profile, although perversely the half-life was now too long, at over 48 hours.

The group thus sought to reduce the half-life by introducing so called 'soft centres' for metabolism into the molecule. This included sequentially incorporating alkyl groups at various positions within the molecule, for oxidation by cytochrome P450 enzymes, and maintaining or improving the biological activity.

Compound **iii** (R = Me) was identified with an IC_{50} value of 2.1 nM (compared to 4.2 nM for compound **ii**) as the racemate [2]. Separation of the isomers, by chiral HPLC of the pyridine precursor to the pyridine N-oxide, gave enantiomer A (IC_{50} = 1.3 nM) and enantiomer B (IC_{50} = 23 nM). Enantiomer A of compound **iii** – L826,141 – was shown to have a single metabolite, the hydroxy methyl compound R = CH_2OH, when incubated in human and rat liver microsomes, and was found to have a shorter half-life in animal models ($t_{1/2}$ = 10 h in rat and 19 h in dog). L826,141 is active in inhibiting bronchoconstriction, both early- and late-phase responses, in animal models, including the sheep and squirrel monkey, by intravenous or oral administration. The compound does not produce emesis in the ferret at 30 mg kg^{-1} oral administration, which was a problem with first generation PDE4 inhibitors.

1 Guay, D. (2002) Discovery of L-791,943: A potent, selective, non emetic and orally active phosphodiesterase-4 inhibitor. *Bioorg. Med. Chem. Lett.* 12, 1457–1461
2 Frenette, R. (2002) Substituted 4-(2,2-Diphenylethyl)pyridine-N-oxides as phosphodiesterase-4 inhibitors: SAR study directed toward the improvement of parmacokintic parameters. *Bioorg. Med. Chem. Lett.* 12, 3009–3013

A lipoprotein-associated phospholipase A_2 inhibitor for evaluation in man

The lipase lipoprotein-associated phospholipase A_2 (Lp-pLA_2) is predominantly associated with low-density lipoprotein (LDL) and catalyzes the hydrolysis of oxidatively modified LDL to lysophospatidyl choline and oxidized fatty acids. Both products are known to be pro-inflammatory and are implicated in atherosclerosis. There is a positive correlation between levels of Lp-pLA_2 and coronary events in hyper-cholesterolemic men.

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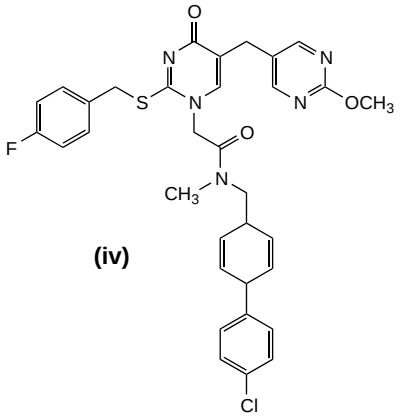
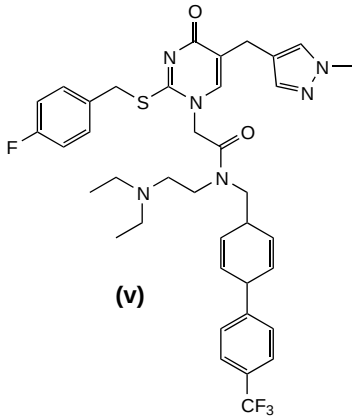


(iv)

A series of letters over the last couple of years from the GlaxoSmithKline (http://www.gsk.com) Medicines Research Centre have detailed the progress of identifying inhibitors of Lp-pLA$_2$ to assess its potential as a target for therapeutic intervention. For example, compound **iv** is a highly potent inhibitor of Lp-pLA$_2$ *in vitro* (IC$_{50}$ = 1 nM) and shows 40% inhibition in human whole plasma at a concentration of 10 nM [3].

The compounds in this series were, however, difficult to formulate for *in vivo* studies and the group chose to replace the N-methyl amide with groups that were designed to improve the aqueous solubility; for example, carboxylic acids, alcohols, amines and amides [4]. The tertiary amine **v** was found to be extremely potent (IC$_{50}$ = 0.06 nM *in vitro* and 3 nM in whole human plasma) and inhibited Lp-pLA$_2$ within the atherosclerotic plaque (74 ± 9%, 30 mg kg^{-1} dose) of the WHHL (Watanabe hereditable hyperlipidaemic) rabbit. Furthermore,



(v)

compound **v** inhibited the formation of lysophosphatidyl choline during the copper catalyzed oxidation of human LDL (IC$_{50}$ = 23 nM) and prevented the subsequent chemotaxis of monocytes (IC$_{50}$ = 10 nM).

The substitution of a chloro group by a trifluoromethyl moiety gave an acceptable CYP450 inhibition profile (IC$_{50}$ > 10 μM against a panel of enzymes) anticipated to minimize drug–drug interactions. The molecule has a reasonable oral bioavailability; 13% in rat, and 24 ± 7% in dog.

Based on this and further data, compound **v** (SB435495) has been selected for clinical evaluation in man.

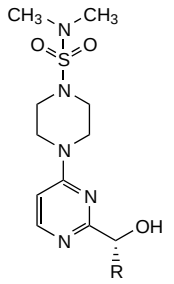
3 Smith, S.A. (2002) Potent, orally active inhibitors of lipoprotein-associated phopspholipase A2: 1-(biphenylmethylamidoalkyl)-pyrimidones. *Bioorg. Med. Chem. Lett.* 12, 51–55

4 Smith, S.A. (2002) The discovery of SB-435495: A potent, orally active inhibitor of lipoprotein-associated phospholipase A2 for evaluation in man. *Bioorg. Med. Chem. Lett.* 12, 2603–2606

Long acting sorbitol dehydrogenase inhibitors to treat diabetic complications

In patients with diabetes, prolonged elevated levels of blood glucose leads to tissue degeneration manifested as neuropathy, retinopathy and so on. A recent hypothesis proposes that excess glucose flux through the polyol pathway gives rise to an imbalance in the NADH:NAD$^+$ ratio and this reductive stress could be the underlying cause of the observed tissue degeneration. The polyol pathway involves the conversion of glucose to sorbitol, followed by the oxidation of sorbitol to fructose – catalyzed by sorbitol dehydrogenase (SDH) – with the concomitant conversion of NAD$^+$ to NADH.

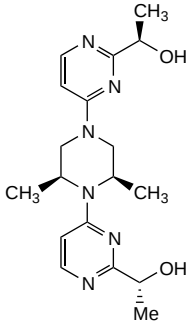
Sorbitol dehydrogenase is a zinc-containing enzyme, and a group from Pfizer Global R&D (http://www.pfizer.com) has described the improvement in potency of the known SDH inhibitor (compound **vi**; IC$_{50}$ = 240 nM) upon



(vi) R = H
(vii) R = CH$_3$

incorporation of a methyl group to give the secondary alcohol (**vii**) with *R*-stereochemistry shown (IC$_{50}$ = 27 nM) [5]. The methyl group conformationally restricts the hydroxymethlene side chain to more effectively coordinate the required zinc atom through the oxygen and pyrimidine nitrogen atoms.

The half-life of the molecule is short because of N-demethylation of the sulfonamide and the group sought to improve the pharmacokinetic and pharmacodynamic properties by replacing the sulfonamide with heterocycles. Compound **viii** was identified with good *in vitro* and *in vivo* potency (IC$_{50}$ = 10 nM and ED$_{90}$ = 2 mg kg^{-1}) in a chronic model of reducing fructose accumulation in the sciatic nerve of streptozotocin-induced diabetic rat [6]. The compound has four chiral centers, however, which is a problem for pharmaceutical development. Also, compound **viii** is rapidly metabolized in rats to give a nearly equal mixture of the four possible isomers about the hydroxyethyl group (*RR, RS, SR, SS*) through



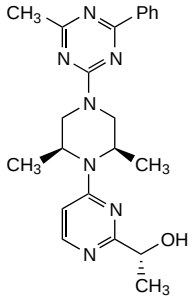
(viii)

oxidation to the aldehyde, followed by reduction. The *RR*-isomer shown is the most active.

The group thus sought to identify improved inhibitors with the ability to reduce the elevation of fructose levels in the sciatic nerve for prolonged periods and only containing the one essential chiral hydroxyethylene group [7]. The ideal compound would have sufficient lipophilicity to partition from the blood to the nerve tissue and also a reduced basicity compared to compound **viii** (pKa = 6.8 and 6.0) to give a greater proportion of the molecule in a neutral form.

Compound **ix** was identified with good *in vitro* potency (IC_{50} = 5 nM) and a dramatic improvement in activity in the chronic rat model (ED_{90} = 0.05 mg kg^{-1}). The molecule has reduced basicity (pKa = 6.2 and 4.8) and increased lipophilicity, having a logP of 2.0 compared to 1.4 for compound **viii**. Compound **ix** appeared to be stable to the oxidoreduction of the chiral hydroxyethylene group by liver microsomes and exhibited a long half-life of 7 h and 10 h in dog and rat, respectively.

The compound is thus anticipated to provide a sustained inhibition of SDH and help clarify the significance of the polyol pathway in the development of diabetic complications.



(ix)

5 Myalari, B.L. (2001) Sorbitol dehydrogenase inhibitors (SDIs): A new potent, enantiomeric SDI, 4-[2-1*R*-hydroxy-ethyl)-pyrimidin-4-yl]-piperazine-1-sulfonic acid dimethylamide. *J. Med. Chem*. 44, 2695–2700

6 Chu-Moyer, M.Y. (2002) Orally-effective, long-acting sorbitol dehydrogenase inhibitors: Synthesis, structure-activity relationships and *in vivo* evaluation of novel heterocycle-substituted piperazino-pyrimidines. *J. Med. Chem*. 45, 511–528

7 Myalari, B.L. (2002) A sorbitol dehydrogenase inhibitor of exceptional *in vivo* potency with along duration of action: 1-(*R*)-{4-[4-(4,6-dimethyl[1,3,5]triazin-2-yl)-2*R*,6*S*-dimethylpiperazin-1-yl]pyrimidin-2-yl}ethanol. *J. Med. Chem*. 45, 4398–4401

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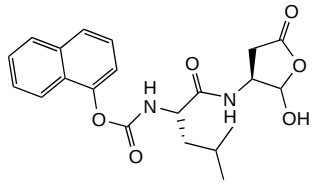
Combinatorial chemistry

Caspase inhibitors

Caspases are a family of cysteine proteases that are involved in both cytokine maturation and apoptosis. Caspase-1 [interleukin-1β (IL-1β) converting enzyme (ICE)] is involved in the induction of inflammation by catalyzing the cleavage of the pro-form of IL-1β. Other caspases have a role in the regulation of apoptosis, either as signalling molecules or as downstream effectors. Inhibition of caspases, either broad spectrum or caspase specific, could be of therapeutic value in the treatment of inflammatory and degenerative diseases, such as rheumatoid arthritis, Parkinson's disease and myocardial infarction. A series of novel, potent, broad spectrum inhibitors has been reported [1].

A library of 46 aspartyl aldehyde compounds was prepared on solid phase. Compounds were designed to probe the SAR in the S3 caspase subunit and were evaluated for their caspase inhibitory activity. Several potent compounds were obtained, one of the most potent being compound **i**, which possessed an IC_{50} value of 570 nM against Csp-1, 132 nM against Csp-3, 940 nM against Csp-6 and 770 nM against Csp-8. This work has produced potent compounds with broad spectrum affinity for caspases 1, 3, 6 and 8, and this class of compounds warrants further investigation.



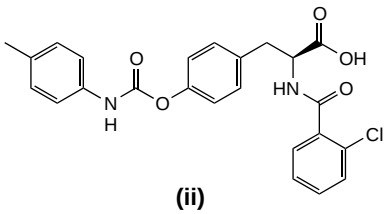
(i)

1 Linton, S. D. *et. al.* (2002) Acyl dipeptides as reversible caspase inhibitors. Part 1: Initial lead optimisation. *Bioorg. Med. Chem. Lett.* 12, 2969–2971

$\alpha_4\beta_1/\alpha_4\beta_7$ Integrin antagonists

Integrins are heterodimeric proteins, which, when expressed in leukocytes, mediate their recruitment to sites of inflammation in a tissue-specific manner. The binding of integrins to surface expressed endothelial proteins initiates cell–cell contacts, which eventually lead to the extravasation of the leukocyte into the tissue.

It has been proposed that improper control of integrin expression can result in pathologies that are directly attributable to the particular expression of the molecules involved. Interaction of the integrin $\alpha_4\beta_7$ with mucosal addressin cell adhesion molecule (MAdCAM) has been implicated in ulcerative colitis and inflammatory bowel disease. The interaction between $\alpha_4\beta_1$ and vascular cell adhesion molecule (VCAM) is thought to contribute to asthma, multiple sclerosis and other autoimmune diseases. Inhibition of these protein–protein interactions significantly effects animal models of disease. This biological connection to disease has increased the interest in the development of small-molecule antagonists for these integrins. Research has been conducted to identify potent inhibitors of a_4b_7 antagonists [2].



(ii)

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