DDT Vol. 6, No. 6 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.

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Molecules

Orally active inhibitors of lipoprotein-associated phospholipase A_2 for the potential treatment of atherosclerosis

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is able to hydrolyze oxidatively-modified low-density lipoprotein (LDL) to generate lysophosphatidyl choline and oxidized fatty acids. Both products are known to be proinflammatory and have been implicated in atherosclerosis. The inhibition of Lp-PLA_2 is therefore a potentially attractive target for the treatment of atherosclerosis.

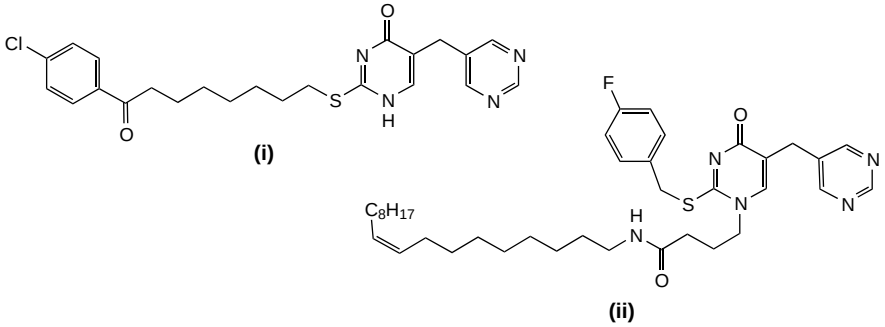
Molecule **(i)** had previously been identified as a potent, non-covalently binding inhibitor of Lp-PLA_2 by a group at SmithKline Beecham (Harlow, UK), with an IC_{50} value of 54 nM, but low activity in plasma. In an effort to synthesize molecules with *in vivo* activity, SAR studies suggested that substitution of the nitrogen in the pyrimidone ring would give improved activity in plasma. Further modification afforded molecule **(ii)** with improved potency (IC_{50} = 0.4 nM), and it was found to exhibit 85% inhibition of Lp-PLA_2 at a concentration of 100 nM in human plasma[1]. Molecule **(ii)** is orally active in a rabbit model dosed at 10 mg kg^{-1}, with a duration of action >5 h, and could aid the elucidation of the role of Lp-PLA_2 in the atherosclerotic process.

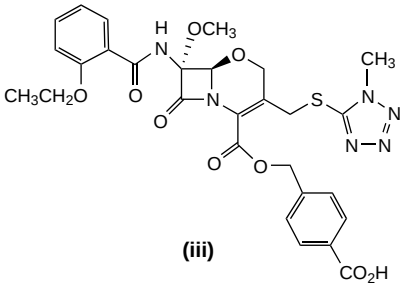
1 Smith, S.A. *et al.* (2000) *N*-1 Substituted pyrimidin-4-ones: novel orally active inhibitors of lipoprotein-associated phospholipase A_2. *Bioorg. Med. Chem. Lett.* 10, 2557–2561

1-Oxacephem based human chymase inhibitors

Chymase is a serine protease stored in the secretory granules of mast cells. Its function is uncertain, although it is known to cleave angiotensin I to angiotensin II, participate in histamine release, activate interleukin-1β and cleave progelatinase B. Thus, it might play an important role in cardiovascular diseases and chronic inflammation.

Screening for inhibitors of chymase by Shionogi Research Laboratories (Osaka, Japan) identified a 1-oxacephem originally prepared in an antibacterial program. Subsequent optimization generated a highly potent (K_i = 6 nM) and selective inhibitor (α-chymotrypsin 160 nM) of chymase[2]. Precedent suggested that the mechanism of inhibition is the nucleophilic opening of the β-lactam ring by the catalytic serine, forming an acyl enzyme intermediate. However, the molecule is rapidly degraded in plasma (half-life = <10 min) and the major degradative route is believed to be cleavage of the lactam ring. The group needed to balance the two conflicting features of chemical reactivity leading to inhibitor potency, but also metabolic lability. Molecule **(iii)** was subsequently identified as a potent inhibitor (K_i = 27 nM), with moderate stability in plasma (half-life = 1.5 h), as a candidate for *in vivo* assays, which should aid our understanding of the physiological function of chymase and its potential as a therapeutic target[3].





2 Aoyama, Y. *et al.* (2000) Synthesis and structure–activity relationships of a new class of 1-oxacephem-based human chymase inhibitors. *Bioorg. Med. Chem. Lett.* 10, 2397–2401

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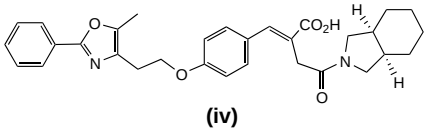
3 Aoyama, Y. *et al.* (2000) 1-Oxacephem-based human chymase inhibitors: discovery of stable inhibitors in human plasma. *Bioorg. Med. Chem. Lett.* 10, 2403–2406

Dual acting insulin sensitizer and insulin secretagogue

Non-insulin dependent diabetes mellitus (NIDDM) is characterized by insufficient secretion of insulin from the pancreas and resistance to insulin in peripheral tissues. Combination therapy of insulin sensitizers and insulin-stimulating agents has been reported to exert a synergistic anti-hyperglycaemic effect. A group at the Welfide Corporation (Osaka, Japan) sought to combine these properties in a single molecule. Intrigued by structural similarities between molecules with insulin-sensitizing activity and insulin-stimulating agents, the group set out to incorporate the key structural features in a hybrid molecule. Compound **(iv)** was identified, which exhibits comparable insulin-releasing activity to Nateglinide and insulin-sensitizing activity to Pioglitazone, both being representative examples of their respective mechanistic class. This is the first reported dual agent and it will be interesting to see the progress of this class of compounds *in vivo*.



(iv)

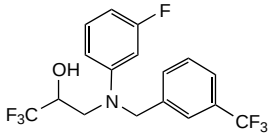
4 Kitajima, H. *et al.* (2000) Hybridization of non-sulphonylurea insulin secretagogue and thiazolidinedione-derived insulin sensitizer. *Bioorg. Med. Chem. Lett.* 10, 2453–2456

Inhibitors of cholesteryl ester transfer protein

Epidemiological studies have shown that high levels of high-density cholesterol (HDL-C), so-called 'good' cholesterol, correlate with a low incidence of coronary heart disease. Cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl ester from high-density lipoprotein (HDL) to low and very low-density lipoprotein (LDL and VLDL, respectively) with a balanced exchange of triglyceride. Thus, CETP converts the protective HDL-C into the pro-atherogenic LDL and VLDL.

A study of CETP polymorphisms identified an allele in men associated with reduced CETP activity, elevated HDL-C and a lowered incidence of coronary heart disease (CHD). Small-molecule inhibitors of CETP could, therefore, be expected to reduce the risk of coronary heart disease.

Screening by a group at Pharmacia (St Louis, MO, USA) identified the amino propanol **(v)** as a weakly active (IC_{50} = 40 μM) inhibitor of CETP in a buffered assay[5]. Exploring the SARs of this series identified **(vi)** as a potent (IC_{50} = 0.02 μM) inhibitor that is also active in human serum (IC_{50} = 0.6 μM). The (*R*) isomer shown is more active than the (*S*) (IC_{50} = 0.08 μM), the absolute configuration being confirmed from the crystal structure of a related molecule. Compound **(vi)** reversibly inhibits the CETP-mediated transfer of triglyceride and cholesteryl ester. Furthermore, it is selective, because it inhibits neither lecithin cholesterol acyl transferase nor phospholipid transfer protein. It will be interesting to observe the progress of this class of compounds.



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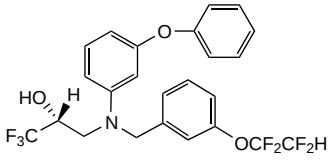


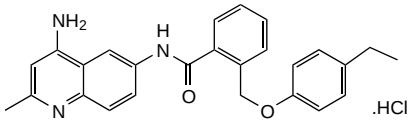
(vi)

5 Durley, R.C. *et al.* (2000) Discovery of chiral *N*,*N*-disubstituted trifluor-3-amino-2-propanols as potent inhibitors of cholesteryl transfer protein. *J. Med. Chem.* 43, 4575–4578

Small-molecule nociceptin antagonists with analgesic activity

Nociceptin is a heptadecapeptide and the endogenous agonist of the G-protein-coupled receptor, opioid receptor-like-1 (ORL_1). ORL_1 mediates the inhibition of adenylate cyclase and has a similar amino acid sequence to opioid receptors, although it does not bind ligands selective to μ-, δ- or κ-opioid receptors. The physiological role of nociceptin is unknown; however, its pharmacological effects include hyperanalgesia and allodynia (pain response provoked by a normally innocuous stimulus). A group at JT (Osaka, Japan) set out to identify a small-molecule antagonist, with CNS penetration, to further evaluate the nociceptin-ORL_1 receptor system and assess its therapeutic potential[6].

Screening identified a series of 4-aminoquinoline derivatives and SAR studies determined the structural requirements for binding, subsequently leading to optimization of potency and bioavailability, exemplified by compound **(vii)**.



(vii)

Molecule **(vii)** is a full antagonist with a K_i value of 2.6 nM, and is active in several animal models after oral administration. In mice, it was found to antagonize nociceptin-induced allodynia and exhibited an analgesic effect in a hot-plate test. In rats, molecule **(vii)** exhibited an analgesic effect in a formalin test, which, unlike morphine, was not antagonized by naloxone (a narcotic antagonist).

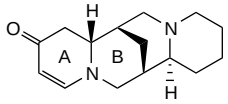
Molecule **(vii)**, therefore, represents a novel class of analgesic and has been chosen for progression into clinical trials.

6 Shinkai, H. *et al.* (2000) 4-Aminoquinolines: novel nociceptin antagonists with analgesic activity. *J. Med. Chem.* 43, 4667–4677

Quinolizin-2-one derivatives as novel hypoglycaemic agents

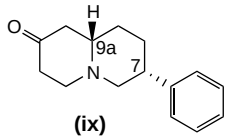
Diabetes, a disease that causes high levels of mortality worldwide, appears as two forms, namely: insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM). Although insulin is the most effective drug for both types, it has drawbacks, mostly associated with its way of administration. Therefore, new effective drugs are highly desirable.

The alkaloid (–)-multifluorine **(viii)**, is known to have hypoglycaemic activity[7]. In addition, the plants from which it is isolated, namely *Lupinus hirsutus* and *Lupinus termis* (leguminosae), are present in the traditional medicine for the treatment of diabetes.



(viii)

Recently, (–)-multifluorine has been chosen as a lead compound by researchers[8] in a project that aims to determine the essential structural requirements of non-insulin dependent hypoglycaemic agents. In particular, the researchers assumed that rings A and B of **(viii)** would be responsible for its activity. On these bases they synthesized a series of quinolizin-2-one derivatives, which were preliminary screened on STZ-induced diabetic mice. The most interesting compound was (7R*,9aS*)-7-phenyl-octahydroquinolizin-2-one **(ix)**.



(ix)

When further investigated in a glucose tolerance test in normal mice, **(ix)** demonstrated a hypoglycaemic effect ~fourfold higher than the model **(viii)**. It should be noted that the stereochemistry at the C-7 of **(ix)**, similar to that of (–)-multifluorine, plays an important role. Indeed, its inversion led to a completely inactive compound. The quinolizidin-2-one ring system is different from conventional drugs used in the treatment of diabetes. Therefore, it could potentially be considered in the development of novel agents for the treatment of both NIDDM and IDDM.

7 Murakoshi, I. *et al.* (1992) Japanese Patent N° 4295480

8 Kubo, H. *et al.* (2000) The hypoglycaemic effect of (7R*,9aS*)-7-phenyl-octahydroquinolizin-2-one in mice. *Biol. Pharm. Bull.* 23, 1114–1117

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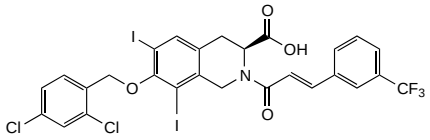
Combinatorial chemistry

Inhibition of protein phosphatase cdc25B

Protein phosphatase cdc25B is a member of a specific family of protein phosphatases that catalyze the dephosphorylation and activation of cyclin-dependent kinases. This step is necessary for cells to undergo mitosis. Inhibition of cdc25B could prove to be therapeutically useful as a treatment for cancer. A solid-phase parallel synthesis approach was used for lead optimization of a template discovered from HTS of the Pharmacia & Upjohn (Peapack, NJ, USA) research compound collection, which possessed inhibitory activity against protein phosphatase cdc25B (Ref. 1). Two libraries containing a total of 75 individual compounds were synthesized on Wang solid-phase resin. The compounds were tested for inhibition against protein phosphatase cdc25B. Several compounds were identified with equivalent or better activity compared with the initial lead compound identified from HTS.

One of the most potent compounds isolated, which was resynthesized and purified before being rescreened, was **(i)**, which possessed an IC_{50} of 23 μM against cdc25B. This work might, therefore, prove to be useful in the further optimization of the lead compounds identified in this library for the production of even more potent and novel inhibitors of protein phosphatase cdc25B.

1 Fritzen, E.L. *et al.* (2000) The solid phase synthesis of tetrahydroisoquinolines having cdc25B inhibitory activity. *Bioorg. Med. Chem. Lett.* 10, 649–652



(i)

N- and P/Q-type calcium channels

Voltage-sensitive calcium channels control various biological processes, such as neurotransmitter release and muscle contraction, and are classified on the basis of electrophysiological properties as L-, N-, P/Q-, R-, or T-type calcium channels. Although their molecular basis has been established in terms of diversity of the α_1 subunit, the contribution of each channel to a total calcium current is often dissected pharmacologically by using specific blockers. ω-Conotoxins, derived from the venom of marine *Conus* snails, are one of the most prominent among various classes of blockers, particularly for blocking N- and P/Q-type calcium channels. ω-Conotoxin GVIA and ω-conotoxin MVIIA bind to N-type channels and ω-conotoxin MVIIC binds to P/Q-type channels with high affinity, and to N-type channels with low affinity. The N- and P/Q-type calcium channels regulate neurotransmitter release in presynaptic nerve terminals and play distinct roles in the nervous system;

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