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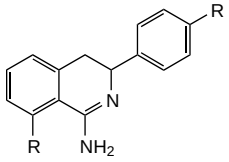
Monitor: molecules and profiles

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.

Molecules

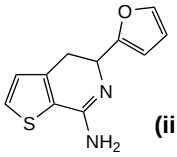
Nitric oxide synthase inhibitors with isoform selectivity

Nitric oxide (NO) is a short-lived, potent cell-signaling agent. The enzymes responsible for the synthesis of NO from L-arginine are the nitric oxide synthases (NOSs). Three isoforms have so far been characterized, of which two are constitutive and are found in nervous or endothelial tissue. These are known as neuronal nitric oxide synthase (nNOS) and endothelial nitric oxide synthase (eNOS), respectively. The third, inducible nitric oxide synthase (iNOS), is able to produce NO in much higher cytotoxic concentrations. Overexpression of iNOS is implicated in certain inflammatory diseases, such as rheumatoid arthritis. Overstimulation of nNOS has been linked with certain neurological diseases, whereas inhibition of eNOS would be detrimental as it has an important role in vascular homeostasis.



(i) R = H
(ii) R = F

A group at AstraZeneca (Charnwood, UK) identified the dihydroisoquinoline derivative **(i)** as a modest inhibitor of iNOS (IC_{50} 9 μM) and nNOS (1 μM), with desirable selectivity over eNOS (inactive at 100 μM) (Ref. 1). It was found that little substitution was allowed, suggesting that the core structure is close to the maximum size of the enzyme active-site. The difluoro analogue **(ii)** was tolerated and gave a significant increase in potency, as well as maintaining selectivity (IC_{50} values are; iNOS, 0.16 μM, nNOS, 16 μM, eNOS inactive). Reduction in ring size, to alleviate possible steric clashes, gave the more potent compound **(iii)** (IC_{50} values are; iNOS, 0.09 μM, nNOS, 0.04 μM, eNOS, 0.92 μM; Ref. 2).



(iii)

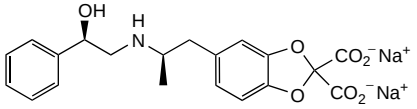
Reduction in potency was observed in a move to cell-based assays because of the high concentration of arginine (~1 mM). However, compounds **(ii)** and **(iii)** exhibited a great improvement over the standard inhibitor *N*-monomethyl arginine (NMMA) [IC_{50} 83 μM for **(ii)**, 2.2 μM for **(iii)** and 170 μM for NMMA in intact DLD-1 cells].

Compound **(iii)** was evaluated *in vivo* and was shown to reduce iNOS-catalyzed production of NO in a rat lipopolysaccharide model. However, compound **(iii)** also increased mean arterial blood pressure, a measure of eNOS inhibition, and was thus shown to be insufficiently selective *in vivo* to be taken further.

1 Hamley, P. *et al.* (2001) 3,4-Dihydro-1-isoquinolinamines: A novel class of nitric oxide synthase inhibitors with a range of isoform selectivity and potency. *Bioorg. Med. Chem. Lett.* 11, 1023–1026
2 Hamley, P. *et al.* (2001) Thienopyridines: Nitric oxide synthase inhibitors with potent *in vivo* activity. *Bioorg. Med. Chem. Lett.* 11, 1027–1030

Azolidinediones as potent and selective human β_3-agonists

The β_3-adrenergic receptor (β_3-AR) has been shown to have various physiological effects, including lipolysis and thermogenesis in white and brown adipose tissue, respectively. Therefore, there is interest in identifying selective agonists for the β_3-AR as potential agents in the treatment of obesity and diabetes.



(iv)

The β_3-AR agonist **(iv)**, discovered in the laboratories of Wyeth-Ayerst (Pearl River, NY, USA), was shown to be an effective anti-obesity and anti-diabetic agent in rodent models. Clinical trials were, however, disappointing because of species differences between the rodent and human receptor. Thus, the group cloned the human receptor and expressed it in a Chinese hamster ovary cell line.

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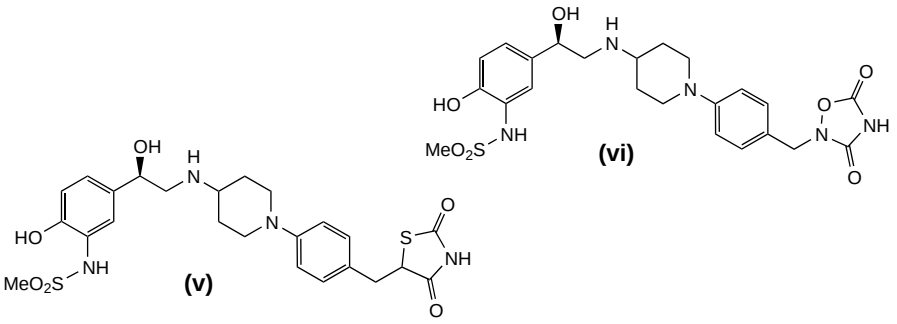


This facilitated the discovery of the thiazolidinedione **(v)**, a potent and selective human β_3-AR agonist (EC_{50} 0.01 μM for the β_3-AR, compared with 1.2 μM for the β_2-AR and 2.7 μM for the β_1-AR; Ref. 3). The compound was also shown to be only a weak antagonist for the β_1- and β_2-receptors. Selectivity is essential because any β_1-AR or β_2-AR agonism would lead to increased heart rate or muscle tremors, which are both unacceptable in a drug candidate.

Administration of compound **(v)** to human β_3-AR transgenic mice showed a thermogenic effect of +30% (±4%), compared with −2% (±4%) in β_3-AR knockout mice. Variation of the thiazolidinedione ring identified the oxadiazolidinedione **(vi)** as a more selective agonist (EC_{50} = 0.02 μM for β_3-AR; 259- and 745-fold more active over the β_2- and β_1-receptors, respectively[4]). Molecule **(vi)** also exhibited a thermogenic effect *in vivo* and was shown to have no activity at the peroxisome proliferator-activated receptor-γ.

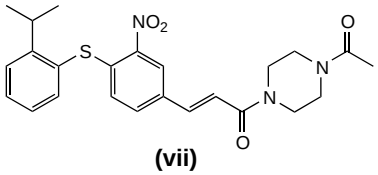
3 Hu, B. *et al*. (2001) 2,4-Thiazolidinediones as potent and selective human β_3 agonists. *Bioorg. Med. Chem. Lett.* 11, 757–760

4 Hu, B. *et al*. (2001) New oxadiazolidinedione derivatives as potent and selective human β_3 agonists. *Bioorg. Med. Chem. Lett.* 11, 981–984

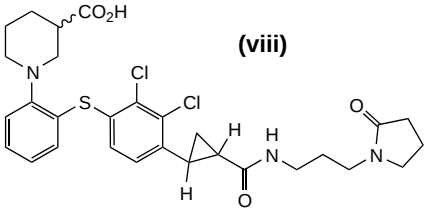
Diarylsulfide cyclopropylamides as antagonists of the LFA-1–ICAM-1 interaction

An early step in the inflammatory process is the adhesion of circulating leukocytes to the vascular endothelium in the region of a tissue injury. The process is mediated by a protein–protein interaction between leukocyte function-associated antigen-1 (LFA-1) on the leukocyte, and intercellular cell-adhesion molecule-1 (ICAM-1) on the endothelium. Disruption of this interaction could provide a means of suppressing this early inflammatory response.

A group conducting metabolic disease research from Abbott Laboratories (Abbott Park, IL, USA) has previously identified the trans-cinnamide diarylsulfide **(vii)** as a potent small-molecule antagonist of the LFA-1–ICAM-1 interaction (IC_{50} = 44 nM; Ref. 5). Because of concerns about the metabolic stability of the cinnamide, the team wanted to identify a suitable replacement and considered the trans-cyclopropylamide[6]. A further issue was the large drop in potency in cell-based assays in the presence of plasma protein.



The polarity of each region of the molecule was varied in an attempt to reduce plasma-protein binding and provide sufficient aqueous solubility. Compound **(viii)** was identified as a diastereomeric mixture about the carboxyl group, which exhibited an IC_{50} value of 10 nM in the absence of serum, and 29 nM in the presence of serum. This represented a drop in activity of less than fivefold in the presence of plasma protein, an improvement on the loss in activity of two orders of magnitude experienced with earlier compounds.



Compound **(viii)** exhibited an oral bioavailability of 27% and a half-life of 1.2 h in the rat. These values are not optimal, but suggest that a suitable molecule might be derived from related structures.

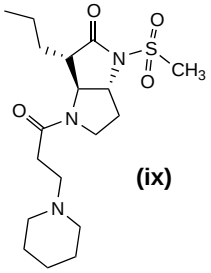
5 Liu, G. *et al*. (2000) Discovery of novel *p*-arylthio cinnamides as antagonists of leukocyte function-associated antigen-1/intracellular adhesion molecule-1 interaction. 1. Identification of an additional binding pocket based on an anilino diaryl sulfide lead. *J. Med. Chem*. 43, 4025–4040

6 Link, J.T. *et al*. (2001) Discovery and SAR of diarylsulfide cyclopropylamide LFA-1/ICAM-1 interaction antagonists. *Bioorg. Med. Chem. Lett.* 11, 973–976

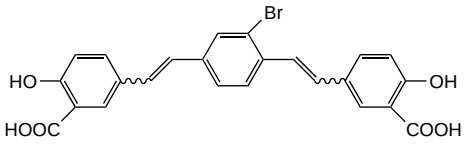
Orally bioavailable neutrophil elastase inhibitor

Human neutrophil elastase (HNE) is a serine protease whose inhibition is of interest for the treatment of respiratory diseases, including emphysema and chronic bronchitis.

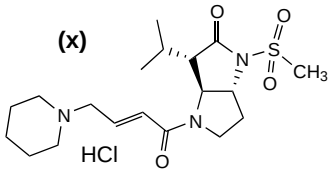
Researchers at the GlaxoSmithKline medicines research centre (Stevenage, UK) have previously described compound **(ix)** as an effective irreversible inhibitor of intracellular HNE (Ref. 7). The racemic compound **(ix)** is orally active, although it is cleared rapidly by opening of the lactam ring, and the required oral dose is high. Converting the n-propyl group to isopropyl, to provide greater steric protection of the carbonyl to nucleophilic attack, gave the desired effect, improving stability in human blood from 3.5 h for **(ix)** to >6 h for the isopropyl derivative[8]. The introduction of a double bond into the amide chain provides greater conformational rigidity and resulted in an increase in potency [IC_{50} = 26 nM for compound **(x)**



(ix)



(xi)



(x)

as the racemate, compared with 120 nM for compound **(ix)**]. One enantiomer, shown, is much more potent than the other (IC_{50} = 22 nM and 274 nM, respectively). Oral bioavailability was found to be close to 100% in the dog with a half-life of 1.5 h and clearance of 28 ml min^{-1} kg^{-1}. A dose of 2 mg kg^{-1} was found to abolish circulating HNE activity, maintaining >90% inhibition for four days. This is believed to be a result of irreversible inhibition of intracellular HNE in neutrophils in the bone marrow and the subsequent release of cells containing pre-inhibited HNE. Therefore, the duration of action is independent of half-life. The pre-incubation of neutrophils with compound **(x)** did not affect their ability to kill a range of infectious organisms. Compound **(x)**, GW311616A, has been selected as a development candidate.

7 Macdonald, S.J. *et al.* (2001) Intracellular inhibition of human neutrophil elastase by orally active pyrrolidine-*trans*-lactams. *Bioorg. Med. Chem. Lett.* 11, 243–246

8 Macdonald, S.J. *et al.* (2001) The discovery of a potent, intracellular, orally bioavailable, long duration inhibitor of human neutrophil elastase – GW311616A, a development candidate. *Bioorg. Med. Chem. Lett.* 11, 895–898

Styrylbenzene derivatives as novel probes for amyloid plaques in the brain

It is widely recognized that β-amyloid (Aβ) peptides are involved in the development and progression of Alzheimer's disease (AD). The prevention and reversal of Aβ-plaque formation are, therefore, targeted as a possible treatment for this disease. Ligands that exhibit specific affinity for Aβ plaques could be useful for both the diagnosis and treatment of AD (Ref. 9). The known ligands for staining amyloid aggregates are usually based on highly conjugated dyes [e.g. Congo red (CR), thioflavins, chrysamine G (CG) and X34][10,11]. In addition, (*E,E*)-1-bromo-2,5-*bis*-(3-hydroxycarbonyl-4-hydroxy)-styrylbenzene [BSB; *E,E*-1; compound **(xi)**] was evaluated as a fluorescent probe for AD and found to have high sensitivity and specificity, and it crosses the blood–brain-barrier[12]. Recently, Kung and coworkers[13] have undertaken the synthesis of all four isomers of BSB, to investigate the influence of geometry on its binding affinity.

Surprisingly, all the isomers displayed strong inhibition of [^{125}I]-(*E,E*)-1-iodo-2,5-*bis*-(3-hydroxycarbonyl-4-methoxy)-styrylbenzene (IMSB) binding to $A\beta_{1\text{-}40}$ aggregates (1–40 indicates the length of the peptide; at peptide concentrations of 10–20 nM). Inhibition constants (K_i) of *E,E*-1 (BSB), *E,Z*-1, *Z,E*-1, and *Z,Z*-1 were 0.11 ± 0.01, 0.19 ± 0.03, 0.27 ± 0.06, and 0.13 ± 0.02 nM, respectively, whereas CG had a K_i value of 0.14 ± 0.04 nM. Moreover, in brain sections of confirmed AD cases, all four isomers showed fluorescent staining that was comparable with, or better than, that of thioflavin S at a similar concentration (0.05 mM). Because isomerization of the compounds was possible under the assay conditions, the kinetics for the conversion of *Z,Z*-1 to *E,E*-1, which is the most probable conversion, was evaluated by NMR studies under strong basic conditions at elevated temperatures. As the activation energy was determined to be 14.15 kcal mol^{-1}, no conversion appears possible at room temperature.

When the nature of the interactions between these small ligands and the macromolecular Aβ aggregates is elucidated, it will be possible to design novel ligands with a high affinity for Aβ plaques.

9 Selkoe, D.J. (2000) Imaging Alzheimer's amyloid. *Nat. Biotechnol.* 18, 823–824

10 Klunk, W.E. *et al.* (1994) Small molecule β-amyloid probes which distinguish homogenates of Alzheimer's and control brains. *Biol. Psychiatry* 35, 627

11 Klunk, W.E. *et al.* (1998) Chrysamine G, a lipophilic analogue of Congo red, inhibits Aβ-induced toxicity in PC12 cells. *Life Sci.* 63, 1807–1814

12 Skovronsky, D. *et al.* (2000) *In vivo* detection of amyloid plaques in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 97, 7609–7614

13 Lee, C.W. *et al.* (2001) Isomerization of (*Z,Z*) to (*E,E*)1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)-styrylbenzene in strong base: probes for Amyloid plaques in the brain. *J. Med. Chem.* 44, 2270–2275

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