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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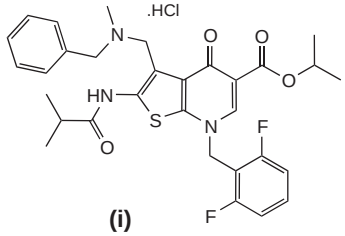
Molecules

Orally active non-peptide antagonist for the human luteinizing hormone-releasing hormone

Luteinizing hormone-releasing hormone (LHRH) is a decapeptide that regulates reproductive function by stimulating the synthesis and release of luteinizing hormone and follicle-stimulating hormone. These, in turn, regulate the synthesis of gonadal steroids and gametes.

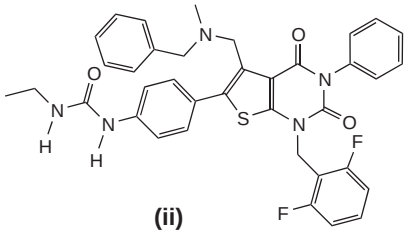
Peptide LHRH agonists are used clinically for the treatment of endocrine-based diseases, including prostate and breast cancer, endometriosis and precocious puberty. They act via a receptor down-regulation mechanism to suppress gonadal steroid synthesis thus achieving biochemical castration. A serious side-effect of this form of treatment is the initial surge of related hormones, known as the 'flare effect', that actually exacerbates the symptoms initially.

Antagonists of LHRH would be expected to suppress gonadotropins from the onset and this has been shown for peptide LHRH antagonist. Compound **i**



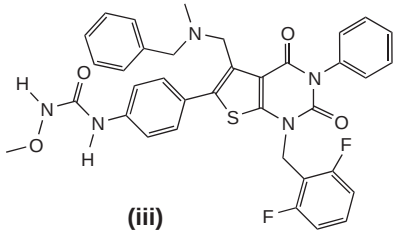
(i)

has been described as a highly potent non-peptide LHRH antagonist [1]. However, when given orally it was not as effective as anticipated and this prompted scientists from the pharmaceutical division of Takeda Chemical Industries (http://www.takeda.co.jp) to search for small-molecule LHRH antagonists with improved oral bioavailability and efficacy [2]. One strategy was to replace the thienopyrimidine-4-one scaffold with other heterocycles, which led to the identification of thienopyrimidine-2,4-dione as a core template, as exemplified in compound **ii**.



(ii)

SAR investigation of substituents around the heterocyclic nucleus was progressed using an assay to measure inhibition of (^{125}I)leuprorelin binding. Functional antagonism was determined by measuring the inhibition of LHRH-stimulated release of arachidonic acid from LHO cells expressing the LHRH receptor. Compound **ii** exhibited a binding affinity (IC_{50}) of 0.1nM and a functional activity of (IC_{50}) of 0.07nM, both for the human receptor.



(iii)

The oral absorption of urea (compound **ii**) was found to be low in the cynomolgus monkey (C_{max} = 0.063 μM and $AUC_{0\text{-}6}$ = 0.27 μm h^{-1} at 10 mg kg^{-1}). Ureas are well known to have poor oral absorption and solubility characteristics due to their strong H-bonding potential.

The methoxy urea (compound **iii**) showed similar activity to the urea (IC_{50} 0.1 and 0.06 nM binding and functional antagonism, respectively), yet showed improved oral bioavailability (C_{max} = 0.21μM, AUC = 0.85 μm h^{-1}). The oxygen of the methoxy urea is believed to form an intra-molecular H-bond, stabilizing the *cis* conformation of the urea shown and thus shielding the hydrogen bonding moieties and reducing the energy cost for desolvation required for intestinal absorption.

The effectiveness of compound **iii** *in vivo* was demonstrated by its ability to suppress the elevated concentration of plasma luteinizing hormone in castrated male cynomolgus monkeys. Oral administration at 10 mg kg^{-1} gave effective and prolonged suppression to 20% of pre-treatment levels from 8–24 h.

1359-6446/03/$ – see front matter PII: S1359-6446(02)02721-1

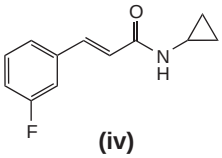
Compound **iii** has been selected for clinical evaluation for the treatment of sex-hormone-dependent pathologies.

1 Cho, N. (1998) Discovery of a novel, potent and orally active non-peptide antagonist for the human luteinizing hormone-releasing hormone receptor. J. Med. Chem. 41, 4190–4195
2 Sasaki, S. (2003) Discovery of a thieno[2,3-d]pyrimidine-2,4-dione bearing a p-methoxyureidophenyl moiety at the 6-position: A highly potent and orally bioavailable non-peptide antagonist for the human luteinizing hormone-releasing hormone receptor. *J. Med. Chem*. 46, 113–124

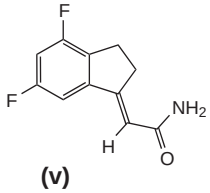
Indanylidenes: anti-inflammatory and analgesic muscle relaxants

Back pain is a debilitating condition for a great number of people and is frequently treated with non-steroidal anti-inflammatory agents and sometimes additionally with muscle relaxants. However, the muscle relaxants currently prescribed can cause drowsiness.

Compound **iv** has been shown to be a centrally acting muscle relaxant that does not cause drowsiness. It was withdrawn from clinical trials and a group from GlaxoSmithKline (http://www.gsk.com) investigated the more rigid cyclic indanylidene structure as a potential backup series, as exemplified by compound **v** [3].



(iv)



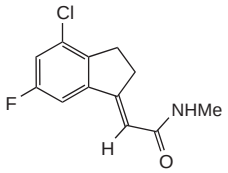
(v)

The compounds were evaluated for their ability to cause muscle relaxation in a mouse morphine-induced Straub tail (ST) assay – a measure of centrally acting agents. The sedative effects of the compounds were estimated in a mouse rotorod (RR) assay. The ratio of the activities (RR:ST) measured as ED_{50} in mg kg^{-1} post oral (po), gave an indication of the dose proportions required to provide muscle relaxation in comparison to sedation.

Compound **iv** had an ED_{50} value of 156 mg kg^{-1} in the ST assay and 189 mg kg^{-1} in the RR assay, giving an RR:ST ratio of 1.2. Compound **v** had an improved profile with higher potency in the ST assay (ED_{50} = 69 mg kg^{-1}) and a superior RR:ST ratio of 2, which would be less likely to promote drowsiness at dose levels producing muscle relaxation.

Compound **v** was also found to have potent anti-inflammatory and analgesic properties, which would be clearly advantageous for agents intended to treat lower back pain. In *in vivo* assays for anti-inflammatory activity (based upon rat carrageenan-induced pleurisy), compound **v** compared favourably with ibuprofen both in reducing tissue exudates (ED_{50} value of 15 mg kg^{-1} compared with 8 mg kg^{-1} po) and in reducing inflammatory cell accumulation (ED_{50} value of 19 mg kg^{-1} compared with 34 mg kg^{-1} po).

It is interesting to note that the muscle relaxant activity in this series can be reduced while retaining potent anti-inflammatory and analgesic activity. Compound **vi** is essentially inactive in the ST and RR assays, however, it exhibits ED_{50} values of 8 and 5 mg kg^{-1} for cells and edema in the carrageenan-inflammation assay and 1.4 mg kg^{-1} in the trypsin hyperalgesia assay [4]. Compound **vi** would thus be expected to be a potent analgesic and anti-inflammatory without causing drowsiness or muscle relaxation.



(vi)

The mechanism of action of these compounds is unknown. Their analgesic properties are not attenuated by naloxone, an opiate antagonist, suggesting that they are not acting through opiate receptors. Compound **v** does inhibit monoamine oxidase reversibly (IC_{50} values of 0.2 μM and 11 μM for MAO-B and -A, respectively), but does not specifically inhibit arachidonate metabolizing enzymes, such as cyclooxygenase, nor does it affect a host of other receptors tested.

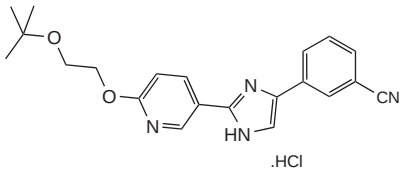
Compound **v** is thus anticipated to have a desirable side-effect profile and, as such, entered Phase I clinical trials where it does not cause drowsiness up to 250mg kg^{-1} oral dosing.

3 Musso, D.L. (2003) Indanylidenes. 1. Design and synthesis of (*E*)-2-(4,6-difluoro-1-indanylidene)acetamide, a potent, centrally acting muscle relaxant with antiinflammatory and analgesic activity. *J. Med. Chem*. 46, 399–408
4 Musso, D.L. (2003) Indanylidenes. 2. Design and synthesis of (*E*)-2-(4-chloro-6-fluoro-1-indanylidene)-N-methylacetamide, a potent anti-inflammatory and analgesic agent without centrally acting muscle relaxant activity. *J. Med. Chem*. 46, 409–416

Characterization of a selective NPY5 receptor antagonist

Neuropeptide Y (NPY) is a highly conserved 36-amino-acid peptide that exhibits a potent, centrally mediated orexigenic effect of appetite stimulation. There are at least five NPY G-protein coupled receptor subtypes and the NPY5 is believed to be the dominant receptor subtype regarding food intake. Evidence for this includes the correlation of appetite stimulation with binding and functional activity of NPY5 peptide agonists. Selective NPY5 antagonists might therefore have potential as anorectic agents, reducing appetite and food intake. As such, they could have a role in the treatment of obesity.

A group from the cardiovascular and metabolic diseases department at Pfizer (http://www.pfizer.com) identified a series of 2,4-diarylimidazoles as NPY5 antagonists from HTS and subsequent optimization identified compound **vii**



(vii)

[5]. This molecule has a high binding affinity for the NPY5 receptor of humans (K_i 1.2 nM) and rats (K_i 1.7 nM) and is a potent antagonist of Ca^{2+} mobilization in a stably transfected cell line (IC_{50} = 0.4 nM). It is also highly selective (NPY1 and NPY2 IC_{50} values >1μM), showing no significant activity in a panel of >50 enzymes and receptors.

The compound is sufficiently well exposed in both the peripheral and central nervous system upon oral administration (C_{max} values of 4.4 μM in plasma, 8.5 μM in brain, and 0.2 μM in cerebrospinal fluid, at 30 mg kg^{-1} po) to be used as a probe for investigating NPY5 antagonism and the effects on feeding in the rat. Compound **vii** was administered orally to Sprague-Dawley rats followed by a selective NPY5 agonist – bovine pancreatic peptide (BPP) – given ICV. Compound **vii** reduced BPP-induced food intake by 55%, but did not produce significant changes in food consumption in either starved rats or naturally feeding rats. Neither did it exert a thermogenic effect or cause changes in locomotor activity.

These results and similar findings with structurally distinct NPY5 antagonists suggest that NPY5 blockade does not produce anorexia in rodents under normal physiological conditions. The precise role of the NPY5 receptor remains to be discovered.

5 Elliot, R.L. (2003) In vitro and in vivo characterisation of 3-{6-(2-tert-butoxyethoxy)pyridine-3-yl]-1H-imidazol-4-yl}benzonitrile hydrochloride salt, a potent and selective NPY5 receptor antagonist. *J. Med. Chem.* 46, 399–408

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