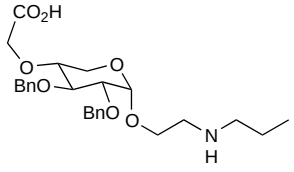
Combinatorial chemistry

Carbohydrate-based peptidomimetics

Dysfunction of integrin-mediated adhesion is often related to cancer metastasis, angiogenesis and osteoporosis. The most biologically relevant $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$ integrins recognize the short Arg-Gly-Asp (RGD) peptidin sequence exhibited by their protein ligands, fibronectin, vitronectin and other proteins of the extracellular matrix. A major problem with inhibition of adhesion is to achieve this with selectivity of a protein *vis-à-vis* with a particular integrin. This selectivity is thought to be related to a bioactive conformation of the RGD sequence and could be specific for its receptor. Chapleur and coworkers have designed novel selective RGD mimics based on chiral scaffolds using a combination of computer-aided design and a solution-phase combinatorial approach for compound synthesis[1]. A library of 126 mimetics of the RGD sequence based on the D-xylose sugar scaffold was synthesized in solution as mixtures of 14. These mixtures were then tested by estimating the adhesion of S180 sarcoma cells, which express only the $\alpha_V\beta_3$ integrin, on a substrate of fibronectin or vitronectin in the presence of the compounds, and compared with the effect of the RGDS peptide as reference on the same cells. Active mixtures identified were iteratively deconvoluted, followed by resynthesis of individual compounds to determine activity. One of the most potent compounds discovered was **(i)**, which gave a percentage inhibition of approximately 45% in this assay, which was identical to the RGDS peptide. This library has been successful in identifying compounds with moderate activity as antagonists of the $\alpha_V\beta_3$ integrin, and could be useful in the future for the construction of other biologically relevant libraries of peptidomimetics, and also in the design of synthetic receptors using parallel synthesis.



(i)

1 Chapleur, Y. *et al.* (2001) Design, synthesis and preliminary biological evaluation of a focused combinatorial library of stereodiverse carbohydrate-scaffold-based peptidomimetics. *Bioorg. Med. Chem. Lett.* 9, 511–523

Neuropeptide FF antagonists

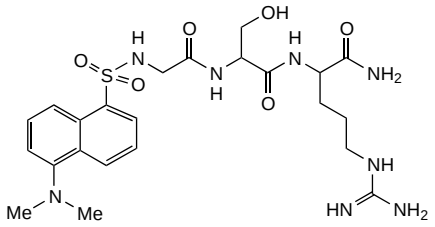
Opiate tolerance, dependence and abuse represent major medical and social problems. Neuropeptide FF (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2), together with the related mammalian neuropeptides NPAF and the N-terminally extended compound **(ii)**, have been identified as a high affinity endogenous ligand for a novel neuropeptide Y-like human orphan G-protein-coupled receptor, HLWAR77. Neuropeptide FF is an anti-opioid and has been implicated in pain modulation, morphine tolerance and morphine abstinence. Antagonists of **(ii)**, besides their importance as pharmacological agents helpful in defining the physiological and pharmacological role of the endogenous neuropeptide, could enable the management of withdrawal symptoms that adversely affect the treatment of opiate abuse. Prokai and coworkers are searching for novel antagonists of **(ii)** that show improved potency and also retain the ability to cross the blood–brain barrier (BBB) (Ref. 2). A library of 741 compounds was synthesized in mixtures of 19 on a Rink amide-methoxybenzyl-hydrazine (MBHA) resin (AnaSpec, San Jose, CA, USA). Screening of these mixtures in a rat spinal-cord membrane preparation for displacement of [^{125}I]YLFQPQRF-NH_2 **(iii)** gave several active mixtures. Mixtures containing glycine (G), lysine (L) and glutamine (Q) showed the highest increase in the percentage displacement of **(iii)** upon screening. Following deconvolution of

Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH-Ala-Gln-Ser

(ii)

[^{125}I] Tyr-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2

(iii)



(iv)

the active mixtures, one of the most active compounds obtained was **(iv)**, which gave a measured K_i value in the radioligand-binding assay of 1.4 ± 0.5 μM. This work has provided moderately potent antagonists of **(ii)**, which are able to cross the BBB, and thus lays the foundation for the design of more potent inhibitors in the future.

2 Prokai, L. *et al.* (2001) Combinatorial lead optimization of a neuropeptide FF antagonist. *J. Med. Chem.* 44, 1623–1626

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Profile

Glycine transporter GlyT-2 blockers: potential pain-relief and anti-spastic drugs

The amino acid glycine is a major neurotransmitter, active in both excitatory and inhibitory synapses in the brain and the spinal cord. The inhibitory actions of glycine are mediated by a strychnine-sensitive glycine receptor, which is a glycine-gated chloride ion channel. Opening of the chloride ion channel following binding of glycine to its receptor results in membrane hyperpolarization,

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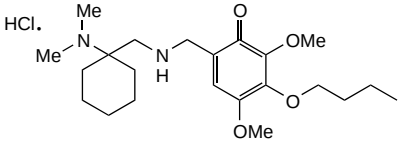
so that the firing of the post-synaptic neuron is inhibited. Glycine is also a co-agonist at excitatory glutamate synapses, acting on postsynaptic *N*-methyl-D-aspartate (NMDA) receptors. The synaptic levels of glycine at both synapse types are controlled by high-affinity glycine transporters located at the presynaptic membranes. The transporters pump released glycine back into the presynaptic neuron, thereby terminating the glycine signal. The glycine transporters belong to a large family of sodium or chloride-dependent transporters, which include the serotonin, dopamine and norepinephrine transporters, and are composed of single oligomeric proteins containing 12 hydrophobic membrane-spanning domains[1].

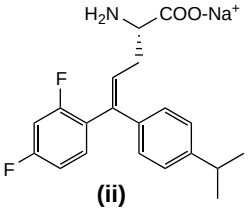
Gene cloning studies have identified two glycine transporters: type 1 (GlyT-1) and type 2 (GlyT-2). The GlyT-1 transporter is widely distributed throughout the CNS, and is co-localized with NMDA-type glutamate synapses, in which glycine acts as a co-agonist for glutamate. The GlyT-2 transporter is confined to the spinal cord and the brain stem region, and is correlated with inhibitory glycine synapses[2].

The brain stem and spinal cord inhibitory glycine synapses are implicated in nociception, in which the action of glycine inhibits neurotransmission and can, therefore, relieve pain. Moreover, glycine-mediated inhibition induces muscle relaxation, whereas the blockade of glycine receptors by strychnine induces convulsions. Blocking the action of the GlyT-2 transporter would allow higher synaptic levels of glycine to accumulate, thereby enhancing glycine-mediated inhibition at these synapses. The GlyT-2 transporter is, therefore, a drug target for potential muscle-relaxant, pain-relief, and analgesic drugs. A wide range of chronic diseases, from neuropathic pain to epilepsy and Parkinson's disease, can be considered as potential future markets for selective GlyT-2 transporter blockers.

Selective inhibitors of the GlyT-2 transporter, such as those reported recently by Organon (OSS, The Netherlands)[3] and NPS Pharmaceuticals (Salt Lake City, UT, USA)[4], are lead compounds for new potential pain-relief drugs. Organon's lead compound is 4-butyloxy-3,5-dimethoxy-*N*-[(1-dimethylaminocyclohexyl)methyl]benzamide **(i)**, a compound with an IC_{50} value of 214 nM at the human GlyT-2 transporter and high selectivity over the human GlyT-1 transporter, as assayed in Chinese hamster ovary (CHO) cells stably expressing these transporters. NPS Pharmaceutical's lead compound **(ii)** contains a 2,4-difluorophenyl group *trans* to the glycine moiety and the *cis*-4-isopropylphenyl substituent, and exhibits an IC_{50} value of 330 nM at the GlyT-2 transporter, with good selectivity over the GlyT-1 transporter.



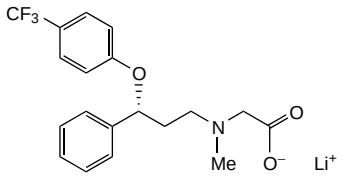
(i)



(ii)

The race is also on to identify selective blockers for the GlyT-1 transporter. Such selective blockers could enhance glutamatergic transmission at NMDA-type receptors by elevating the synaptic concentrations of glycine, the co-agonist for glutamate at the NMDA receptor. This glutamate receptor subtype is implicated in cognition[5]. Hence, selective GlyT-1 transporter blockers could be potential cognition-enhancing drugs for treating Alzheimer's disease patients. A recent study from Organon[6] reports on the identification of such a lead compound, ORG24598 **(iii)**, exhibiting an IC_{50} value of 180 nM at the human GlyT-1 transporter, but also having a low affinity for the human GlyT-2 transporter.

Blockers of another renowned ion-dependent transporter, the serotonin transporter, are in global therapeutic use for treating depression and additional mood disorders, and have generated massive revenues for the pharmaceutical industry since their introduction in the late-1980s. It is, therefore, not surprising that the therapeutic potential of glycine transporter blockers is eagerly followed by the pharmaceutical industry.



(iii)

1 Zafra, F. *et al.* (1997) Molecular biology of glycinergic neurotransmission. *Mol. Neurobiol.* 14, 117–142

2 Jursky, F. and Nelson, N. (1995) Localization of GlyT-2 reveals correlation with the distribution of glycine receptor. *J. Neurochem.* 64, 1026–1033

3 Wilson, L. *et al.* (2001) The first potent and selective inhibitors of the glycine transporter type 2. *J. Med. Chem.* 44, 2679–2682

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