Monitor

After many years as our regular *Combinatorial chemistry* contributor, Nick Terrett has decided to hand on the role. Nick has made an important contribution to *Monitor* over the years and I would like to formally thank him on behalf of all the editorial team and our readership for his support since the early days of *DDT*. I would also like to welcome Paul Edwards, the new *Combinatorial chemistry* columnist, to the *Monitor* team; I am sure all the readers will find his contributions as interesting and stimulating as those previously provided by Nick.

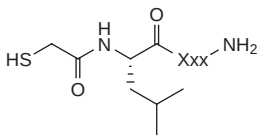
Andrew W. Lloyd
Monitor Editor

Combinatorial chemistry

Matrix metalloprotease inhibitors

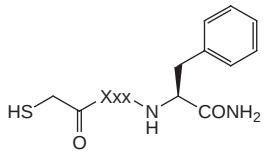
Matrix metalloproteases (MMPs) are a large family of zinc-dependent endoproteases involved in the remodelling of connective tissue. The enzymes can be divided into four main groups: collagenases, gelatinases, stromelysins and membrane-type MMPs. Abnormal expression and regulation of this group of enzymes has been directly implicated in the development of several pathological conditions including rheumatoid arthritis, cancer invasion and metastasis. A combinatorial approach using positional scanning techniques has been used to identify agents with activity against several pathologically important MMPs (Ref. 1).

A small library of 40 compounds were individually synthesized on Rink-amide AM solid support using a positional scan of 20 amino acids at position Xxx of (**i**) and (**ii**), respectively. Several of



(i)

the compounds synthesized provided reasonably potent compounds for MMP-1 or MMP-8 over the other MMPs tested. For example, (**i**) with Xxx = Trp had an IC_{50} for MMP-1 of 50 nM, and



(ii)

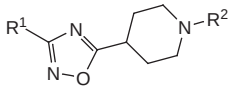
selectivities of 3–79-fold over MMPs 2, 3, 8 and 9. Meanwhile, (**ii**) with Xxx = Nva, possessed an IC_{50} of 240 nM against MMP-8, and selectivities of 0.5–16-fold over other MMPs tested. By screening these compounds, it was possible to identify which peripheral groups made the greatest contribution to binding. This work has enabled the identification of alternative pharmacophores that might be used to focus the design and for the development of new libraries of MMP inhibitors with enhanced selectivity for individual species.

1 Lynas, J.F. *et al.* (2000) Solid-phase synthesis and biological screening of *N*-α-mercaptoamide template-based matrix metalloprotease inhibitors. *Comb. Chem. High Throughput Screen.* 3, 37–41

Selective dopamine D4-receptor ligands

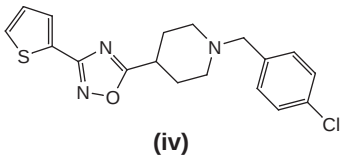
Hyperactivity of the dopaminergic system has long been linked to the aetiology of schizophrenia. Several dopamine-receptor subtypes have been discovered that can be divided into 'D_1-like' (D1, D5) or 'D2-like' (D2, D3, D4) groups. Typical antipsychotics effectively block both D4 and D2 receptors. It is hypothesized that D4-receptor blockade imparts the neuroleptic effect of the typical antipsychotics and the D2-receptor blockade is responsible for the unwanted extrapyramidal side effects. Thus, a selective D4-receptor antagonist would represent an effective treatment for schizophrenia. The use of combinatorial chemistry in the discovery of novel inhibitors selective for D4 over D2 is described in a recent paper[2].

A library of 332 individual oxadiazolylpiperidines based on the core motif (**iii**) was prepared in solution, and the



(iii)

products purified by preparative LC–MS using mass-triggered sample collection. One of the most potent and selective compounds (hD4 versus hD2) isolated from this library was (**iv**), which has a



(iv)

K_d of 5 nM against hD4, with >50-fold selectivity over hD2. The authors have combined combinatorial synthesis techniques with automated purification to deliver, in only one round of synthesis, a new series of selective D4-receptor ligands based on the 1,2,4-oxadiazole bioisostere of the ester group.

2 Williams, J.P. and Lavrador, K. (2000) A solution-phase combinatorial synthesis of selective dopamine D4 ligands. *Comb. Chem. High Throughput Screen.* 3, 43–50

Piperazine-2,5-diones for cytotoxicity assays

Derivatives of piperazine-2,5-diones have shown potential as therapeutic

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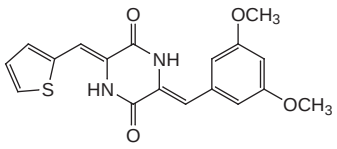
agents and are used in medicine as antibiotics, synthetic vaccines and in cancer therapy. A new 'one-pot' parallel solution-phase combinatorial synthesis method for preparing piperazine-2,5-diones has been developed to enable the rapid identification of potential cytotoxic agents[3]. The approach involved identifying several piperazine-2,5-diones with activity in a brine shrimp lethality assay, used as an antitumour prescreen. A positive correlation exists between the brine shrimp assay and the cytotoxicity assay used for this study, and the brine shrimp assay is accurate in predicting *in vivo* activity as cytotoxicity in a series of human solid-tumour cell lines[4].

A set of 61 piperazine-2,5-diones were individually synthesized in a solution-



(v)

phase combinatorial library, of which (**v**) is an example of one of the most toxic compounds towards brine-shrimp (18 μg ml^{-1} inhibition level). Hence, this compound is a potential lead compound for more specific cytotoxicity assays. Through screening, key side chains that are potential pharmacophores have been identified that could be factored into the next round of library design. Screening of additional analogues of this kind against specific assays could be carried out in the future to eliminate toxic analogues that are not antitumour compounds, and identify analogues that are antitumour compounds.

3 Loughlin, W.A. *et al.* (2000) Solution-phase combinatorial synthesis and evaluation of piperazine-2,5-dione derivatives. *Bioorg. Med. Chem. Lett.* 10, 91–94

4 McLaughlin, J.L. *et al.* (1993) *ACS Symposium Series* 534 (Kinghorn, A.D. and Baladrin, M.F., eds), (Chapt. 9), pp. 112–137, American Chemical Society

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Contribution of NO/$ONOO^-$ pathway to the deleterious effect of traumatic brain injury in mice

Traumatic brain injury (TBI) is a major factor in the mortality of young people in western countries and leads to persistent, long-term neurological dysfunction in survivors. To-date, no available pharmaceutical compound has proven its effectiveness during clinical trials[1]. TBI is a combination of immediate, irreversible, mechanical dysfunction of brain tissue and secondary damages that develop over a period of hours to days following injury. Similarities exist between lesion mechanisms in brain ischaemia and in TBI.

Reports indicate that inhibition of nitric oxide synthases (NOS) by arginine analogues protect against cerebral ischemia[2]. In this context, our group investigated the involvement of the L-arginine–NO pathway in the neurological consequences of TBI by assessing the effect of NOS inhibitors in a closed head injury model in mice[3].

N^{ω}-nitro-L-arginine methyl ester (L-NAME), which exerts a neuroprotective activity in both mice[4] and rat models of focal cerebral ischaemia[5], was first used to inhibit NOS. As L-NAME inhibits both neuronal (nNOS) and non-neuronal NOS isoforms, we also examined the effect of 7-nitroindazole (7-NI), which preferentially inhibits the neuronal isoform[6] and also has a neuroprotective effect in focal cerebral ischemia[7]. Low doses of L-NAME or 7-NI administered shortly after TBI significantly decreased the neurological deficit induced by TBI (Ref. 8). These results, in accordance with Wada[9], suggest that NO synthesis by nNOS plays an important role in the early neurotoxic cascade leading to neurological deficit following TBI.

Increasingly, evidence supports a role for oxygen free radicals (OFR) in the pathophysiology of TBI (Refs. 10,11). Firstly, during TBI in mice, an increased production of OFR was reported[12]. Secondly, these OFR contribute to the neurological damage induced by TBI, as free radical scavengers attenuate post-traumatic pathophysiology and/or promote survival and recovery in experimental head injury[13]. We also reported that α-phenyl-tertbutylnitrone and melatonin, two antioxidants, decrease the neurological deficit induced by TBI (Ref. 14).

NO-related tissue injury might be largely caused by peroxynitrite ($ONOO^-$), generated by the reaction between NO and superoxide[15,16]. One of the actions of peroxynitrite is to nitrate tyrosine residues, leading to protein nitration. Immunohistochemical studies with anti-nitrotyrosine antibody revealed that nitration of tyrosine residues occurs in various tissues and organs under pathological conditions, for example in post-ischaemic heart tissue[17], in the brain after carbon monoxide poisoning[18], and in the brain of patients with Alzheimer's disease[19] or amyotrophic lateral sclerosis[20]. As free radical generation is triggered by TBI, the cytotoxic peroxynitrite could be formed. Nitrotyrosine formation was increased 4 h and 24 h after TBI and was primarily observed in degenerating neurons, in the sites of direct and diffuse impact[21]. Furthermore, L-NAME reduced nitrotyrosine formation and the number of nitrotyrosine-positive neurons, suggesting that reactions mediated by the NO/$ONOO^-$ pathway occur during TBI. Inhibition of NOS might be neuroprotective by reducing NO production and subsequent cytotoxic peroxynitrite generation. Peroxynitrites are deleterious by degrading DNA. Repair of DNA-strand breakages induce excessive activation of poly(ADP-ribose) synthase

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