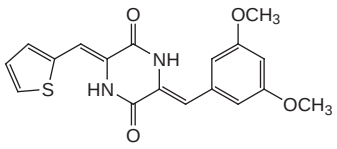
agents and are used in medicine as antibiotics, synthetic vaccines and in cancer therapy. A new 'one-pot' parallel solution-phase combinatorial synthesis method for preparing piperazine-2,5-diones has been developed to enable the rapid identification of potential cytotoxic agents[3]. The approach involved 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.

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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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(PARS), which leads to energy depletion by consumption of β-nicotinamide adenine dinucleotide (the source of ADP-ribose) and ATP.

Inhibitors of PARS exert neuroprotection against traumatic neuronal injury in hippocampal slices subjected to fluid percussion[22] and against ischaemic brain injury in rat pups[23]. We have therefore investigated the effects of the PARS inhibitor 3-aminobenzamide on the neurological consequences of TBI. We showed that PARS inhibitors reduced the neurological deficit elicited by TBI (Ref. 24).

Taken together, these results strongly suggest a deleterious production of NO following TBI. Concomitant production of NO and OFR results in peroxynitrite production participating in the lesion mechanisms. PARS activation could represent one of the mechanisms accounting for peroxynitrite toxicity. These data suggest new therapeutic strategies for the treatment of acute traumatic brain injury.

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Collaboration…

Incyte Genomics (Palo Alto, CA, USA) has expanded its agreement with **Aventis Pharmaceuticals** (Frankfurt, Germany) to focus on identifying individual variations in single nucleotide polymorphisms that affect drug metabolism. This collaboration will include access to Incyte's Custom SNP Program. Roy A. Whitfield, CEO of Incyte, said 'We believe providing customized discovery and analysis of SNPs is invaluable to researchers investigating the link between individual genetic differences and response to medication.'