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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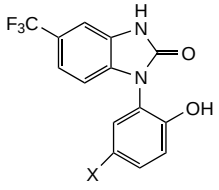
Molecules

3-Aryloxindoles: maxi-K channel openers with neuroprotective properties

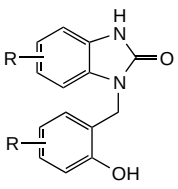
Stroke is currently recognized as the third leading cause of adult disability and death in the USA and Europe. Strokes are classified into two types: ischemic stroke, which results from vessel occlusion, and hemorrhagic stroke, in which there is intracerebral hemorrhage resulting in neuronal damage. Hemorrhagic stroke represents ~20% of all strokes [1]. In an effort to provide clinicians with post-stroke effective neuroprotective therapies, numerous mechanistic approaches have been examined in the past decade, including antagonists of AMPA–kainate [2] and *N*-methyl-D-aspartate (NMDA) excitatory amino acid receptors [3], and inhibitors of neuronal adenosine reuptake [4]. Hewawasam and collaborators [5] have addressed the problem of post-stroke neuroprotection by developing potent and specific openers of large conductance, Ca^{2+}-activated (maxi-K) potassium channels.

Maxi-K channels are of particular interest because of their large channel conductance and their expression in a range of excitable cell types, including neurons and smooth-muscle cells. Therefore, modulators of maxi-K channels are potentially useful agents for the treatment of a variety of disease states associated with both the CNS and



(i) (NS004) X = Cl
(ii) (NS1619) X = CF_3

smooth-muscle function. Among others, the benzimidazolone derivatives NS004 **i** and NS1619 **ii** are prominent prototypes among the small molecule openers of maxi-K channels [6]. In addition, the structural homologs **iii** have been reported (R = H, Cl, CF_3, NO_2, CH_3) [7]. The maxi-K channel-opening activity of 3-(5-chloro-2-hydroxyphenyl)-1,3-dihydro-3-hydroxy-6-(trifluoromethyl)-2*H*-indol-2-one **iv** and related analogs was reported by Hewawasam and shown to be dependent upon the absolute configuration at the stereogenic center [5]. On these bases, the same group has now published a more detailed analysis of SARs of compound **iv** and the structurally related **v** [8]. The effect of the compounds on outward K^+ current was



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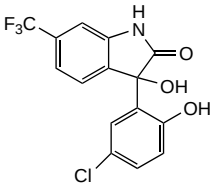

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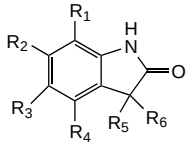

(v)

determined by using a two electrode voltage clamp recording from *Xenopus laevis* oocytes expressing cloned *mSlo* (or *hSlo*) maxi-K channels [9]. Besides **iv**, compound (±)-(**vz**; from a series a–z) (R_1 = R_3 = R_4 = R_6 = H; R_2 = CF_3; R_5 = 2-OH, 5-Cl-C_6H_3) showed interesting properties in this assay. Altogether, the results clearly indicated that channel-opening activity is sensitive to both the nature and the pattern of substitution of both aromatic elements, as well as the absolute configuration of the asymmetric centre.

Subsequent studies conducted on rats, mainly aimed to determine the extent of brain penetration following intravenous administration, demonstrated adequate brain penetration for both (±)-**iv** and (±)-**vz**. However, when the two compounds were tested in a model of focal

stroke that involved a permanent occlusion in the middle cerebral artery (MCAO model), conducted in spontaneously hypertensive rat, only (±)-**vz** demonstrated activity. In particular, intravenous administration of (±)-**vz** at a dose of 0.03 mg kg^{-1} reduced the measured cortical infarct volume by ~18%, compared with the control. In addition, intraperitoneal administration of (±)-**vz** at a dose of 10 mg kg^{-1}, 2 h following artery occlusion, reduced infarct volume by 26%, compared with vehicle-treated control. Under these conditions, the extent of neuroprotection demonstrated by (±)-**vz** was either equivalent or superior to a range of experimental neuroprotectants that possess diverse mechanisms-of-action.

1 Williams, G.R. *et al.* (1999) Incidence and occurrence of total (first-ever and recurrent) stroke. *Stroke,* 30, 2523–2528
2 Goldberg, M.P. *et al.* (1997) Techniques for assessing neuroprotective drugs *in vitro*. *Int. Rev. Neurobiol.* 40, 69–93
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5 Hewawasam, P. *et al.* (1997) Discovery of a novel class of BK channel openers: enantiospecific synthesis and BK channel opening activity of 3-(5-chloro-2-hydroxyphenyl)-1,3-dihydro-3-hydroxy-6-(trifluoromethyl)-2*H*-indol-2-one. *Biorg. Med. Chem. Lett.* 7, 1255–1260
6 Olesen, S-P. *et al.* (1994) Selective activation of Ca^{2+}-dependent K^+ channels by novel benzimidazolone. *Eur. J. Pharmacol.* 251, 53–59
7 Meanwell, N.A. *et al.* (1996) *N*-benzylated benzimidazol-2-one derivatives: activators of large-conductance Ca^{2+}-dependent K^+ channels. *Bioorg. Med. Chem. Lett.* 6, 1641–1646
8 Hewawasam, P. *et al.* (2002) Synthesis and structure-activity relationships of 3-aryloxindoles: a new class of calcium-dependent, large conductance potassium (maxi-K) channel openers with neuroprotective properties. *J. Med. Chem.* 45, 1487–1499
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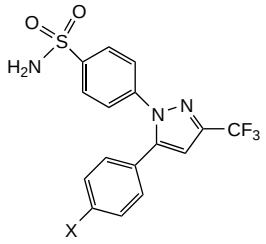
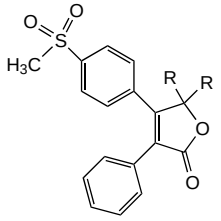
Novel cyclooxygenase-2 selective inhibitors from pharmacophore models

Nonsteroidal anti-inflammatory drugs (NSAIDs) are useful tools in the treatment of inflammation, pain and fever. However, they show undesirable gastric side effects. Because NSAIDs directly target cyclooxygenases (COXs), the discovery of the COX-2 isoform has opened up the possibility of developing COX-2 selective inhibitors to act as an effective NSAID without the gastric side effects. At present, two COX-2 selective inhibitors have successfully reached the market, Celecoxib **vi** [10] and Rofecoxib **viii** [11]. Palomer and collaborators [12] have recently published a series of compounds, which they designed as analogs of the potent but non-selective COX inhibitor indomethacin **x**, using the information available on the tricyclic COX-2 selective **vi–ix**, which all have an arylsulfonyl group that is believed to have a crucial role on their selectivity. In addition, the X-ray crystal structure of COX-2 complexed with SC558 **vii** was considered. The application of the resulting pharmacophore to the design of indomethacin analogs with the basic indole framework, enabled the authors to identify a small set of simple, novel COX-2 selective inhibitors **xi a–d; xii a–c**.

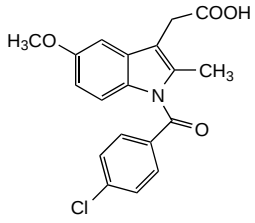
These compounds were tested *in vitro* to verify their inhibition of the PGE_2 generation in lipopolysaccharide-stimulated human monocytes (COX-2 cell assay) and the inhibition of the arachidonic acid (1 μM) induced Tx B_2 generation in isolated human platelets (COX-1 cell assay). Compounds **xia** ($R_1 = CH_3$; $R_2 = H_2$; $R_3 = Cl$), **xid** ($R_1 = CH_3$; $R_2 = H_2$; $R_3 = OCH_3$), and **xiia** ($R_1 = CH_3$; $R_2 = O$; $R_3 = Cl$) showed IC_{50} values towards COX-2 of 0.65 ± 0.26 μM, 0.78 ± 0.58 μM, and 0.73 ± 0.09 μM, respectively, with hardly any effect on COX-1 (IC_{50} >>10 μM). These compounds were additionally tested for their selectivity of COX-2 inhibition in the human whole-blood assay. In this test, compound **xia** inhibited COX-2 in a similar range as the reference compounds **vi** and **viii**. (IC_{50} = 4.3 ± 0.3 μM, 3.6 ± 1.2 μM and 3.4 ± 2.3 μM, respectively). In addition, **xia** showed a favorable COX-1:COX-2 ratio, compared with the same reference compounds (IC_{50} versus COX-1:IC_{50} versus COX-2 = >20, 7.3 and 8.4, respectively).



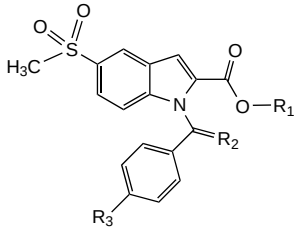
(vi) X = CH_3 (Celecoxib)
(vii) X = I (SC558)



(viii) R = H (Rofecoxib)
(ix) R = CH_3 (DFU)



(x) (Indomethacin)



(xi a–d)
(xii a–c)

10 Penning, T.D. *et al.* (1997) Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzene sulfonamide (SC58635, Celecoxib). *J. Med. Chem.* 40, 1347–1365

11 Ehrich, E. *et al.* (1996) Demonstration of selective COX-2 inhibition by MK-966 in humans. *Arthritis Rheum.* 39, S81 (Abstract 328)

12 Palomer, A. *et al.* (2002) Identification of novel cyclooxygenase-2 selective inhibitors using pharmacophore models. *J. Med. Chem.* 45, 1402–1411

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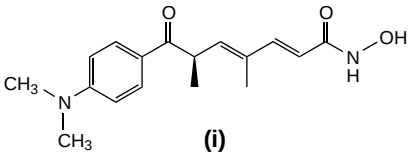
Novel antitumour molecules

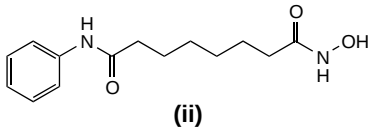
Inhibitors of human histone deacetylase as potential antitumour agents

Reversible acetylation of nuclear histones is a major regulator of gene expression, and cell-specific patterns of gene expression in normal cells result from the balance between histone acetyl transferase (HAT) and histone deacetylase (HDAC) activity. Perturbation of this balance has been linked to cancer and several natural product and synthetic HDAC inhibitors, such as trichostatin A **i** and suberoylanilide hydroxamic acid (SAHA, **ii**) have been reported to have antitumour activity.

Remiszewski and co-workers at the Novartis Institute for Biomedical Research (Summit, NJ, USA) and Westfälische Wilhelms-Universität (Münster, Germany) have reported the synthesis of analogues of SAHA and trichostatin A and their evaluation in a human HDAC enzyme inhibition assay, a $p21^{waf1}$ (p21) promoter assay and in growth inhibitory assays against selected human tumour cell lines *in vitro* [1]. The SAHA analogues prepared displayed comparable activity in the HDAC enzyme inhibition and p21 promoter assays, and among the trichostatin A analogues, enzyme and cellular



(i)



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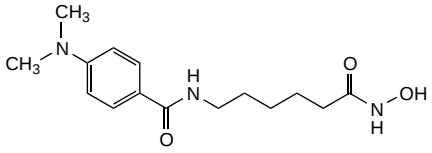


(iii)

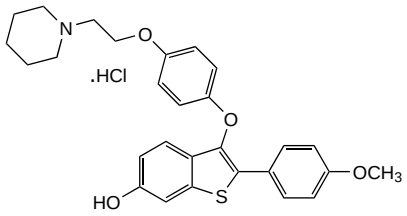
potency was found to be related to chain length, with substitution at the 4-position of the benzamide significantly affecting enzyme potency. Compound **iii** was the most potent enzyme inhibitor among the analogues examined (IC_{50} = 0.046 μM). In addition, compound **iii** was found to affect the growth of a panel of eight human tumour cell lines differentially in the low micromolar IC_{50} range.

1 Remiszewski, S.W. *et al.* (2002) Inhibitors of human histone deacetylase: synthesis and enzyme and cellular activity of straight chain hydroxamates. *J. Med. Chem.* 45, 753–756

A selective oestrogen receptor modulator for chemoprevention of breast cancer

The development of safe chemopreventative strategies for women at high risk from breast cancer is a desirable alternative to the rather drastic surgical intervention through prophylactic bilateral mastectomy. The use of selective estrogen receptor modulators (SERMs) for this purpose has provided important clinical advances in this area through the development of the triphenylethylene tamoxifen and the benzothiophene raloxifene. However, the widespread clinical data on these two agents show that neither is the ultimate agent for breast cancer chemoprevention, either in terms of total efficacy or freedom from undesirable side effects, such as the increased risk of endometrial cancer with prolonged use of tamoxifen.

Sporn and co-workers at the Dartmouth Medical School (Hanover, NH, USA) and Lilly Research Laboratories (Indianapolis, IN, USA) have now reported the development of a new chemopreventative SERM termed arzoxifene **iv** [2]. Arzoxifene was found to be a selective SERM that is a potent estrogen antagonist in mammary and uterine tissue, and also acts as an estrogen agonist to maintain bone density and to lower serum cholesterol. In addition, arzoxifene is a highly effective agent (more potent than raloxifene) for the prevention of mammary cancer induced in the rat by the carcinogen nitrosomethylurea, and was also found to be devoid of the uterotrophic effects of tamoxifen, suggesting that the use of arzoxifene will not increase the risk of endometrial carcinoma.



(iv)

2 Suh, N. *et al.* (2001) Arzoxifene, a new selective estrogen receptor modulator for chemoprevention of experimental breast cancer. *Cancer Res.* 61, 8412–8415

ADEPT for selective cancer treatment: proof-of-principle

The concept of antibody-directed enzyme prodrug therapy (ADEPT) has been known for about the past 15 years, without yet resulting in widespread clinical application. The technique involves the enzymatic conversion of a non-toxic prodrug into a cytotoxic agent at the surface of tumour cells, by employing an enzyme-immuno conjugate, which docks selectively onto the surface of malignant cells prior to administration of the prodrug. Proposed requirements for the prodrug are that the corresponding

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