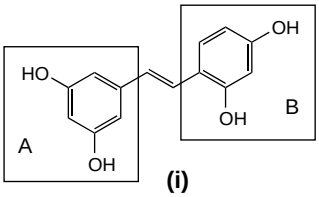
Monitor: molecules and profiles

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.

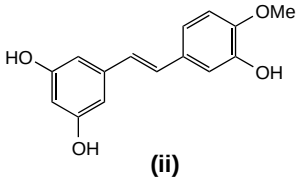
Molecules

Novel *trans*-stilbene analogues as potent and selective human cytochrome P450 1B1 inhibitors

The cytochrome P450s (CYP) catalyze the phase I oxidative metabolism of several xenobiotics and endogenous compounds. The CYP1 subfamily, which includes 1A1, 1A2 and 1B1, has long been of interest because of its relevance to chemical carcinogenesis [1]. In particular, CYP1B1 is known to be a major producer of 4-hydroxyestradiol (4-OHE2). Although the contribution of CYP1B1 and 4-OHE2 in oestrogen-induced cancer is still speculative, increasing evidence for the carcinogenicity of oestrogens suggest that 4-OHE2 is involved in the development of human breast cancer [2]. Therefore, selective inhibitors of CYP1B1 could prevent mammary tumor formation, especially in the breast. In addition, selective inhibitors could also be useful as pharmacological tools to elucidate the functions of CYPs. Several *trans*-stilbene compounds were reported to be inhibitors of CYP, among others, oxyresveratrol **(i)** [3] and rhapontigenin



(i)



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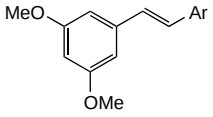

(iii) Ar = 2,4-dimethoxyphenyl
(iv) Ar = 4-methoxyphenyl
(xii) Ar = 2-thiophenyl

(iii–xii)

(ii) [4]. Based on the evidence that previously reported potent CYP1 inhibitors are generally lipophilic, Kim and collaborators [5] have prepared a series of compounds **(iii–xii)** in which the phenyl ring on side A of compounds **(i)** and **(ii)** contains two methoxy groups, instead of the hydroxy groups present in the models. In addition, several structural changes were made on site B, to enhance selectivity.

The inhibitory effects produced by the new compounds on ethoxyresorufin *O*-de-ethylation (EROD) were determined in bicistronic bacterial membranes containing human CYP1A1, CYP1A2 and CYP1B1 [6]. The most selective and potent compound was the tetramethoxystilbene **(iii)**, which was very potent on CYP1B1 (IC_{50} = 6 ± 2 nM) and selective with respect to CYP1A1 and CYP1A2 (IC_{50} = 300 ± 20 nM and 3100 ± 880 nM, respectively). In the same test, compound **(i)** showed IC_{50} values of 15, 150, and 34 μM for 1A1, 1A2, and 1B1, respectively [4]. Compound **(iii)** is a competitive inhibitor of CYP1B1 with a K_i value of 3 nM. It does not cause a time-dependent inactivation of CYP1B1 and it is relatively stable in the presence of CYP1B1. Modification of the methoxy groups of the phenyl ring on site B always resulted in decreased potency and/or selectivity. In particular, it should be noted that compound **(iv)**, which lacks the 2-OMe of **(iii)**, retained the inhibitory activity of the model against 1A1 and 1A2 but was found to be much less active against 1B1 (IC_{50} = 790 ± 100 nM). These results suggest that the presence of a 2-methoxy group could have an important role in binding to the active site of CYP1B1. This hypothesis was confirmed by the observation that when the 2-methoxy was replaced by different substituents, the inhibitory properties of the resulting compounds were always lower, with respect to compound **(iii)**. The only exception was compound **(xii)** (IC_{50} values of 61 ± 21 nM, 11 ± 2 nM and 2 ± 1 nM for 1A1, 1A2 and 1B1, respectively), which lost selectivity.

These results are important to establish the preliminary SARs of stilbenes as CYP1 inhibitors. In addition, the selective compound **(iii)** can be useful to characterize the enzymatic properties of CYP1B1.

1 Ortiz de Montellano, P.R. (1995) *Cytochrome P450: Structure, Mechanism and Biochemistry.* (2nd edn), Plenum Press

2 Spink, D.C. *et al.* (1997) Induction of cytochrome P450 1B1 and catechol estrogen metabolism in ACHN human renal adenocarcinoma cells. *J. Steroid Biochem. Mol. Biol.* 62, 223–232
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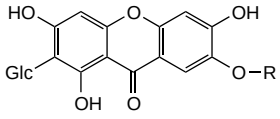
5 Kim, S. *et al.* (2001) Design, synthesis and discovery of novel *trans*-stilbene analogues as potent and selective human cytochrome P450 1B1 inhibitors. *J. Med. Chem.* 45, 160–164
6 Burke, M.D. *et al.* (1985) Ethoxy-, pentoxy- and benzyloxy-phenoxazonones and homologues: a series of substrates to distinguish between different induced cytochromes P450. *Biochem. Pharmacol.* 34, 3337–3345

Antidiabetic activity of the rhizoma of *Anemarrhena asphodeloides*

The rhizoma of *Anemarrhena asphodeloides*, which is one of the constituents of Byakko-ka-ninjin-to (BN), has been used as a traditional Oriental medicine for diabetes and constipation [7]. Although its antidiabetic activity was observed after intraperitoneal administration of its extracts [8], little is known about its oral antidiabetic activity or its active components. Recently, Miura and collaborators [9] have reported the results of a bioassay-guided fractionation of several extracts (water, 50% methanol and methanol) of the rhizoma of *Anemarrhena asphodeloides* (AA) on KK-Ay mice, a type 2 diabetic animal model. The hypoglycaemic effect of the water extract of AA was dose-dependent. At 90 mg kg^{-1}, it lowered blood glucose from the basal level of 570 ± 29 to 401 ± 59 mg l^{-1} (p <0.05), 7 h after oral administration. AA decreased the serum insulin level in KK-Ay mice and decreased blood glucose levels in the insulin tolerance test. By contrast, no change in the mean blood glucose levels was observed in normal mice. These results indicate that the antidiabetic mechanism of AA could be a result of the decreased insulin resistance. AA appears to have little toxicity (LD_{50} >>900 mg kg^{-1}).

The active 50% methanol fraction of AA was analysed by HPLC. Two compounds **(xiii)** were separated, to which the structure of mangiferin (MF) and mangiferin-7-*O*-β-glucoside (MG) were assigned by spectroscopic methods. MF and MG showed similar antidiabetic activity at a dose of 90 mg kg^{-1}.



(xiii)

MF R = H
MG R = Glc
Glc = β-D-glucopyranosyl

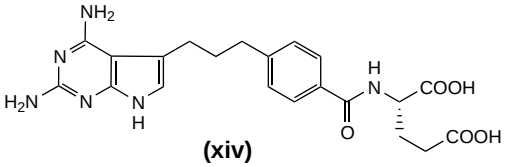
Because little is known about the antidiabetic activity of xantone compounds, these results could open the way to further studies in this field.

7 Kimura, M. *et al.* (1996) Combined potentiating effect of Byakko-ka-ninjin-to, its constituents, rhizomes of *Anemarrhena asphodeloides*, tomosaponin A-III and calcium on pilocarpine-induced saliva secretion in streptozocin-diabetic mice. *Biol. Pharm. Bull.* 19, 926–931
8 Ichik, H. *et al.* (1998) New antidiabetic compounds, mangiferin and its glucoside. *Biol. Pharm. Bull.* 21, 1389–1390
9 Miura, T. *et al.* (2001) Antidiabetic activity of the rhizoma of *Anemarrhena asphodeloides* and active components, mangiferin and its glucoside. *Biol. Pharm. Bull.* 24, 1009–1011

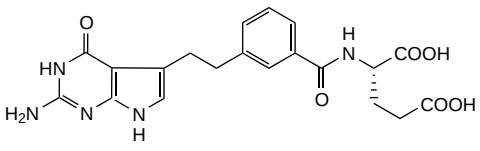
Pyrrolo[2,3-*d*]pyrimidine thymidylate synthase inhibitors

Thymidylate synthase (TS) catalyzes the conversion of 2′-deoxyuridine-5′-monophosphate (dUMP) to 2′-deoxythymidine-5′-monophosphate (dTMP) and is essential for *de novo* DNA biosynthesis. Therefore, TS has long been recognized as an important target for cancer chemotherapy [10]. Among others, compound **(xiv)** was recently reported [11] to exhibit potent antitumor activities against various solid tumors and methotrexate (MTX) resistant mouse lymphocytic leukaemia *in vitro* and *in vivo*. After extensive structural modifications of the pyrrolo[2,3-*d*]pyrimidine antifolates, the 4-oxo compound **(xv)** was found by the same group [12] to moderately inhibit TS (IC_{50} = 4.7 μM). Another group independently published the synthesis and biological properties of compound **(xv)** (LY231514) [13].

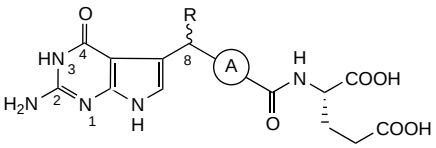
Based on the human TS–dUMP-inhibitor complex model built on the known *Escherichia coli* TS-dUMP-CB3717 ternary complex [14], Aso and collaborators [15] have recently designed several novel pyrrolo[2,3-*d*]pyrimidine derivatives with one-carbon bridge **(xvi–xx)** and reported their inhibitory activity against TS. According to the results of their modelling method, such compounds should possess the essential binding factors, namely three hydrogen bonds (between 2-NH_2 and Ala263, 3-NH and Asp169, and 4-O and Gly173) and the hydrophobic interactions of the benzoyl moiety with the enzyme. The compounds were evaluated against TS purified from mouse fibrosarcoma Meth-A cells (cultured methylcholanthrene-induced sarcoma cells). The amino acid sequence of the mouse enzyme has high homology (91% identity) to the human



(xiv)



(xv) (LY231514)



(xvi–xx)

(xvi)	A = phenyl	R = H
(xvii)	A = 2,5-thienyl	R = H
(xix)	A = phenyl	R = CH_2CH_3

TS sequence and the active site region is exactly conserved. Therefore, it can be assumed that the activity on mouse TS is not significantly different from that of the human TS. Compound **(xvi)** showed better activity (IC_{50} = 1.0 μM) than the model **(xv)** (IC_{50} = 4.7 μM). Replacement of the benzene ring of **(xvi)** by its bioisoster thiophene **(xvii)** gave a 12-fold increase of TS inhibitory potency (IC_{50} = 0.083 μM). Finally, the introduction of a hydrophobic substituent at C8 of **(xvi)** resulted in a 30–60-fold improvement. In particular, compound **(xix)**, which is the most potent of this series (IC_{50} = 0.017 μM), has a 276-fold higher activity than compound **(xv)**.

These results suggest that the substituent at C8 is able to establish a hydrophobic interaction and to fill the unoccupied space created by Glu58, Trp80 and the bridge chain, which had been indicated by docking studies.

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11 Itoh, F. *et al.* (1994) Novel pyrrolo[2,3-d]pyrimidine antifolate TNP-351: cytotoxic effect on methotrexate-resistant CCRF-CEM cells and inhibition of transformylases of *de novo* purine biosynthesis. *Cancer Chemother. Pharmacol.* 34, 273–279

12 Miwa, T. *et al.* (1991) Novel pyrrolo[2,3-d]pyrimidine antifolates: synthesis and antitumor activities. *J. Med. Chem.* 34, 555–560

13 Taylor, E. C. *et al.* (1992) A dideazatetrahydrofolate analogue lacking a chiral center at C-6, N-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid, is an inhibitor of thymidylate synthase. *J. Med. Chem.* 35, 4450–4554

14 Montfort, W. R. *et al.* (1990) Structure, multiple site binding and segmental accommodation in thymidylate synthase on binding dUMP and an anti-folate. *Biochemistry* 29, 6964–6977

15 Aso, K. *et al.* (2001) Pyrrolo[2,3-*d*]pyrimidine thymidylate synthase inhibitors: design and synthesis of one-carbon bridge derivatives. *Chem. Pharm. Bull.* 49, 1280–1287

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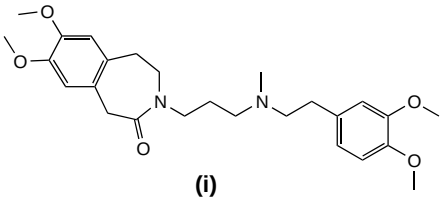
Combinatorial chemistry

I_f channel blockers

Channel-mediated currents that consist of small background currents, such as I_{K1}, I_B and I_f, and large voltage-gated currents, such as I_{ca} and I_K, have an important role in the generation of spontaneous diastolic depolarization and action potential of cardiac pacemaking cells. They can contribute to changes in spontaneous pacemaker activity, affecting slow diastolic depolarization and, hence, the threshold potential for action potential generation and the resulting potential of pacemaker cells. Selective reduction in heart rate with no important changes in contractility and wall tension could have several advantages in the treatment of ischaemic heart diseases.

Zatebradine **(i)** (Boehringer Ingelheim, Ingelheim, Germany) is a representative compound of the therapeutic class of sino-atrial node modulators, which have been shown to inhibit the hyperpolarization activated current I_f [1,2]. The selective blockade of the I_f current causes a reduction of the spontaneous rate of firing of the pacemaker cells without resulting in abolition of pacemaker activity.

The first solid-phase synthesis of Zatebradine and its analogues has been reported [3]. A small library of 21 single analogues was prepared on REM [resin that is (RE)generated following cleavage of the product and is initially functionalized via a (M)ichael reaction] solid phase resin. Upon cleavage, the ability of the library compounds to reduce the spontaneous beating of isolated guinea-pig atria in a concentration-dependent manner was evaluated. One of the most potent compounds isolated was **(ii)**, which showed a maximum reduction of beating of 80% at 3 μM, compared with a reduction of 40% at 3 μM with Zatebradine [compound **(i)**]. This library has resulted in the discovery of a series of compounds with an increased ability to reduce the spontaneous beating of isolated guinea-pig atria. Further studies are required to elucidate the putative actions of these compounds on calcium channels.



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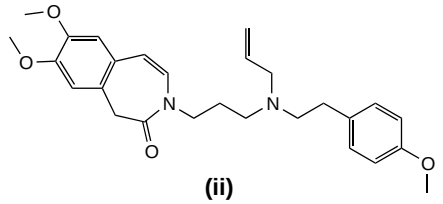

(ii)

1 Reiffen, M. *et al.* (1990) Specific bradycardic agents. 1. Chemistry, pharmacology, and structure-activity relationships of substituted benzazepinones, a-new class of compounds exerting antiischemic properties. *J. Med. Chem.* 33, 1496–1504

2 Bomhard, A. *et al.* (1991) Specific bradycardic agents. 2. Heteroaromatic modifications in the side chain of specific bradycardic benzazepinones: chemistry, pharmacology, and structure-activity relationships. *J. Med. Chem.* 34, 942–947

3 Booth, S. *et al.* (2001) Parallel solid-phase synthesis of Zatebradine analogues as potential I_f channel blockers. *Bioorg. Med. Chem. Lett.* 11, 2351–2354

Thrombin inhibitors

Thrombosis is the result of improper regulation of the hemostasis mechanisms. This leads to the formation of intravascular clots, which could cause tissue damage or cell death because of inadequate blood flow. This cardiovascular disorder

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