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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.

Monitor Editor: Steve Carney

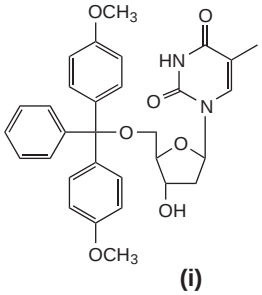
Monitor Authors:
Daniela Barlocco, *University of Milan*
David Barrett, *Fujisawa Pharmaceutical Company*
Paul Edwards, *Pfizer*
Steven Langston, *Millennium Pharmaceuticals*
María Jesús Pérez-Pérez, *Instituto de Química Médica*
Michael Walker, *Bristol-Myers Squibb*
John Weidner, *Emisphere*
Andrew Westwell, *Nottingham University*

Molecules

Mitochondrial thymidine kinase inhibitors

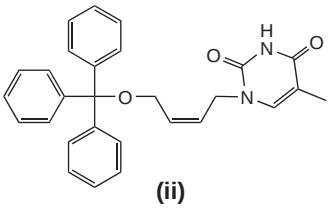
Deoxynucleoside kinases catalyze the phosphorylation of deoxynucleosides to the corresponding deoxynucleoside monophosphates. In mammalian cells, there are four different deoxynucleoside kinases: thymidine kinase-1 (TK-1) and deoxycytidine kinase (dCK), which are present in the cytosol and/or the nucleus, and the mitochondrial enzymes deoxyguanosine kinase (dGK) and mitochondrial thymidine kinase (TK-2). Interest in TK-2 has increased recently owing to its involvement in mitochondrial DNA synthesis and its controversial role in the mitochondrial toxicity that is observed during prolonged treatment with antiviral drugs such as azidothymidine and fialuridine. Moreover, TK-2 is constitutively expressed throughout the cell cycle and represents the predominant fraction of thymidine kinase activity in nonproliferating and resting cells. Therefore, the use of TK-2 inhibitors might help to resolve fundamental questions regarding the physiological role of TK-2 and its contribution to the mitochondrial toxicity of antiviral drugs.

In the search for new TK-2 inhibitors, nucleosides from commercial sources have been tested. Among them, 5′-*O*-(4,4′-dimethoxytrityl)thymidine (DMTr-dThd) (**i**) showed slight inhibitory activity against



(i)

TK-2-catalyzed thymidine phosphorylation ($IC_{50} = 468 \pm 45$ μM) [1]. The presence of the dimethoxytrityl moiety at the 5′ position of thymidine converts the thymidine substrate into an inhibitor because it lacks the 5′-OH group necessary for phosphorylation. Therefore, the sugar moiety was replaced by acyclic spacers and a series of acyclic nucleoside analogues of general formula [Thy]-spacer-[trityl] were synthesized and tested as TK-2 inhibitors. This series was further optimized by replacing or modifying the thymine base and the trityl moiety. Several compounds showed inhibitory activities in the low micromolar range. In particular, the 1-[(*Z*)-4-(triphenylmethoxy)-2-butenyl]thymine (**ii**), a lead compound in this series, showed an inhibitory potency of 1.5 ± 0.16 μM against TK-2 [1]. These compounds constitute the first examples of reversible, non-nucleoside, non-substrate inhibitors of TK-2 that are competitive with respect to thymidine and uncompetitive with respect to ATP [2]. These compounds might be useful for studying the physiological role of the mitochondrial TK-2 enzyme.

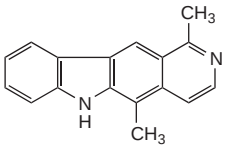


(ii)

1 Hernández, A.I. *et al.* (2002) Acyclic nucleoside analogues as novel inhibitors of human mitochondrial thymidine kinase. *J. Med. Chem.* 45, 4254–4263
2 Balzarini, J. *et al.* (2003) Non-nucleoside inhibitors of mitochondrial thymidine kinase (TK-2) differentially inhibit the closely related herpes simplex virus type 1 TK and the *Drosophila melanogaster* multifunctional deoxynucleoside kinase. *Mol. Pharmacol.* 63, 263–270

A novel aza-bioisoster of the antitumor alkaloid olivacine

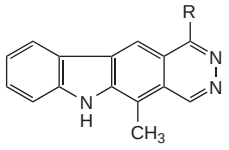
Olivacine (**iii**) is a member of the pyridocarbazole family of alkaloids. It was first isolated from the bark and stem of *Aspidosperma olivaceum* and has been shown to possess significant antitumor activity [3]. The mechanism of action of olivacine is thought to involve stabilization of the 'cleavable complex' formed



(iii)

PII: S1359-6446(03)02644-8

between DNA and the enzyme topoisomerase II [4]. In addition, reactivation of the lost functionality of the p53 protein has been suggested as another mechanism of action for this class of molecules [5]. Recently, the potent olivacine analog S16020-2 [6] was reported to circumvent P-glycoprotein-mediated multidrug resistance [7]. Subsequently, Haider and collaborators reported the synthesis of the 1,5-dimethyl-6*H*-pyridazino[4,5-*b*]carbazole, a 3-aza isoster of olivacine [8]. The compound was prepared in four steps by initial Diels-Alder reaction of 1-methylpyrano[3,4-*b*]indol-3(9*H*)one with 4-oxo-2-pentynoic acid ethyl ester. The cytotoxic activity of the 3-aza-olivacine (**iv**) was tested in four human tumor cell lines using the XTT assay [9]. Preliminary results showed significant activity only at a dose of approximately 10^{-5} M. At lower concentrations (10^{-6} to 10^{-9} M) no dose-dependent activity was seen. The cytotoxic activity of the monomethyl analog (**v**) was less potent in all the tested cell lines.



(iv) R = CH_3
(v) R = H

3 Schmutz, J. *et al.* (1958) Alkaloids of *Aspidosperma olivaceum*. *Pharm. Acta Helv.* 33, 341–347
4 Tewey, K.M. *et al.* (1984) Intercalative antitumor drugs interfere with the breakage–reunion reaction of mammalian DNA topoisomerase II. *J. Biol. Chem.* 259, 9182–9187
5 Ohashi, M. *et al.* (1995) Inhibition of p53 protein phosphorylation by 9-hydroxyellipticine: a possible anticancer mechanism. *Jpn. J. Cancer Res.* 86, 819–827
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7 Pierre, A. *et al.* (1998) Circumvention of P-glycoprotein-mediated multidrug resistance by S16020-2. Kinetics of uptake and efflux in sensitive and resistant cell lines. *Cancer Chemother. Pharmacol.* 42, 454–460
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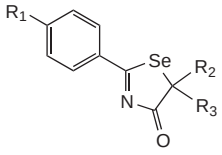
Biological properties of 1,3-selenazol-4-one-derivatives

Selenium is an essential component of several enzymes and has been proposed as a nutritional supplement owing to its potential role as an antioxidant at low concentrations and as an anticancer agent at higher doses [10]. Several selenium-containing heterocycles have been reported to possess biological activity [11,12] and indeed, the synthesis of 1,3-selenazol-4-one derivatives and their inhibitory effects on mushroom tyrosinase were reported recently [13,14]. Tyrosinase is the key enzyme involved in the browning of fruits and vegetables and is also implicated in coloring the skin, hair and eyes in animals. Furthermore, the enzyme is involved in the oxidation of tyrosine to L-dopa (dyhydroxyphenylalanine) and of L-dopa to dopaquinone [15].



(vi) $R_1 = CH_3$, $R_2 = R_3 = H$
(vii) $R_1 = CH_3$, $R_2 = CH_2CH_3$, $R_3 = H$
(viii) $R_1 = R_2 = R_3 = CH_3$
(ix) $R_1 = R_2 = R_3 = H$
(x) $R_1 = Cl$, $R_2 = R_3 = H$
(xi) $R_1 = OCH_3$, $R_2 = R_3 = H$

Compounds **vi** were tested *in vitro* for their ability to inhibit mushroom tyrosinase activity. The tyrosinase inhibitor kojic acid was used as a reference drug. The most potent derivative (**vi**) had an IC_{50} of 333.2 μM and was found to be a competitive inhibitor. In the same assay, kojic acid had an IC_{50} of 934.5 μM. The IC_{50} values of compounds **vii–xi** ranged from 384.3 to >500 μM.

10 Block, E. (1996) Recent results in the organosulfur and organoselenium chemistry of genus *Allium* and *Brassica* plants. Relevance for cancer prevention. *Adv. Exp. Med. Biol.* 401, 155–69
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12 Wu, W. *et al.* (1999) Induction of apoptosis in human gastric adenocarcinoma cells by two novel organoselenium compounds, TS-2 and TS-6. *Anticancer Res.* 19, 5375–5381
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15 Shin, N.H. *et al.* (1998) Oxyresveratrol as the potent inhibitor on dopa oxidase activity of mushroom tyrosinase. *Biochem. Biophys. Res. Commun.* 243, 801–803

María Jesús Pérez-Pérez
Instituto de Química Médica (CSIC)
Juan de la Cierva 3
28006 Madrid, Spain
e-mail: mjperez@iqm.csic.es

Daniela Barlocco
University of Milan
Viale Abruzzi 42
Milano 20131, Italy
tel: +39 02 5031 7515
fax: +39 02 5031 57565
e-mail: daniela.barlocco@unimi.it