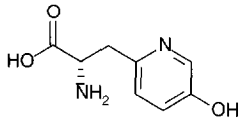
L-Azatyrosine: a new lead in anticancer drug development

It is now known that most, if not all, products of dominantly acting cancer genes are components of cellular signalling pathways and hence targets based on these pathways represent a major area of current interest in antitumour drug development. Ras proteins are guanine-nucleotide-binding proteins which, when bound to GTP, assume an active configuration for signal transduction. Attenuation of the Ras signalling function is achieved via hydrolysis of GTP to GDP by Ras GTPase. Mutated *ras* genes, which encode abnormal variants of low molecular weight G proteins, play a major role in human carcinogenesis, to the extent that they occur in about 40% of human colon cancers and 95% of human pancreatic cancers. Overstimulation of cellular signalling by oncogenic *ras* results because Ras GTPase is not activated in the GTP-bound mutated protein, leading to cell proliferation and differentiation of the kind associated with tumour-cell activity. For a recent review on the role of Ras proteins in signal transduction see Egan, S.E. and Weinberg, R.A. [*Nature* (1993) 365, 781–783]. This profile will focus on the biological activity exhibited by L-azatyrosine, possibly resulting from its effect on oncogenic *ras* genes, and also will discuss recent syntheses of this important molecule.

Origin of L-azatyrosine

β-(5-Hydroxy-2-pyridyl)alanine, or azatyrosine (**1**), was first prepared in racemic form by Norton, S.J., Skinner, C.G. and Shive, W. [*J. Org. Chem.* (1961) 26, 1495–1498], and was found be a potent competitive antagonist of tyrosine in *Leuconostoc dextranicum* 8086 and a moderately active inhibitor of *Escherichia coli* 9723. The antibacterial properties of L-azatyrosine were also described by Inouye, S. and coworkers [*Chem. Pharm. Bull.* (1975) 23, 2669–2677], using optically active material obtained from a fermentation broth of *Streptomyces chibaensis*.



1

Despite these early findings little attention was paid to this area of study until a pioneering report by Shindo-Okada, N. and coworkers [*Mol. Carcinog.* (1989) 2, 159–167] indicated that L-azatyrosine induces permanent reversion of activated c-Ha-*ras*-transformed NIH3T3 cells to the apparently normal phenotype. Importantly, the growth of cells possessing normal *ras* genes does not appear to be significantly affected. The modified amino acid also caused reversion of human cancer cell lines such as pancreatic adenocarcinoma PSN-1 cells, known to have a c-Ki-*ras* mutation, to apparently normal cells.

In vivo activity

A further report by Shindo-Okada, N. and coworkers [*Cancer Res.* (1992) 52, 1628–1630] described the inhibition of chemical carcinogenesis *in vivo* by L-azatyrosine. In these experiments transgenic mice harbouring the human proto-oncogene c-Ha-*ras* were treated with the chemical carcinogen 7,12-dimethylbenz[*a*]anthracene and then L-azatyrosine was applied. The percentage incidence, number per mouse and size of papilloma were found to be greatly reduced compared with tumour growth in the absence of azatyrosine. Irreversible growth suppression of oncogenic *ras*-transformed mouse embryos [Nomura, T. *et al. Jpn. J. Cancer Res.* (1992) 83, 851–858] and *ras*-induced neurite formation in PC12 cells [Shindo-Okada, N. *et al. Oncogene* (1992) 7, 2019–2024] have also been reported. Other important biological effects of L-azatyrosine include the observation by Glickman, J.F. and coworkers [*Proc. Natl. Acad. Sci. U. S. A.* (1992) 89, 7654–7658] that azatyrosine inhibits *ras*-induced maturation of *Xenopus* oocytes [see also Chung, D.L. *et al. Anticancer Res.* (1991) 11, 1373–1378] and also suppresses tyrosine phosphorylation of *Xenopus* mitogen-activated protein kinase in oocytes.

New synthetic routes to L-azatyrosine

The preceding reports describing the important anticancer and antibiotic properties associated with L-azatyrosine coupled to its limited availability in an optically pure form has stimulated the development of several synthetic routes to this important lead compound. Schow, S. and coworkers [*J. Org. Chem.* (1994) 59, 6850–6852] have employed the methodology for the synthesis of optically active amino acids developed by Williams, R.M. and Im, M-N. [*Tetrahedron Lett.* (1988) 29, 6075–6078]. In this procedure, alkylation of the enolate of a chiral glycine equivalent with protected 5-hydroxypicolyl bromide yielded diastereomerically pure protected azatyrosine. Removal of protecting groups gave L-azatyrosine in a five-step overall sequence.

A second recent report concerning the synthesis of L-azatyrosine by Ye, B. and Burke, T.R. [*J. Org. Chem.* (1995) 60, 2640–2641] made use of an organometallic coupling strategy, developed by Jackson, R.F.W. and coworkers [*J. Org. Chem.* (1992) 57, 3397–3404], between an organozinc reagent derived from protected iodoalanine and protected 5-hydroxy-2-iodopyridine. Removal of protecting groups gave L-azatyrosine in a concise overall synthesis. Recently, Myers, A.G. and Gleason, J.L. [*J. Org. Chem.* (1996) 61, 813–815] have reported a highly practical procedure for the preparation of multigram quantities of L-azatyrosine via the highly diastereoselective alkylation of the enolate derived from (*R,R*)-(−)-pseudoephedrine glycinamide with a protected 5-hydroxy-iodomethylpyridine derivative. Again, removal of protecting groups gives a concise overall strategy for the synthesis of optically pure L-azatyrosine. Work within our own group (Stevens, M.F.G. and coworkers, unpublished) provides a concise route to either enantiomer of azatyrosine via the enzymic resolution of a protected α-amino ester.

It is hoped that this short review has demonstrated the potential importance of L-azatyrosine as a lead compound towards the discovery of new selective cancer chemotherapy drugs. It is clear that this molecule and its derivatives will be the subject of much research effort in the near future.

Malcolm F.G. Stevens,
Margaret S. Cooper and
Andrew D. Westwell
Cancer Research Laboratories
Department of Pharmaceutical Sciences,
University of Nottingham
Nottingham, UK

  1359-6446/96/$15.00