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Pyrrolo[2,1-*d*][1,2,3,5]tetrazinones deaza analogues of temozolomide with potent antitumor activity

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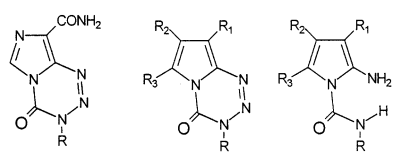
Abstract

The title compounds, that hold the deaza skeleton of temozolomide, exhibited potent in vitro antiproliferative activity. An evaluation of such a biological activity indicates that the mode of action of these compounds differs from that of temozolomide and is also mechanistically unrelated to that of any known antitumor drug. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Pyrrolotetrazines; Azolotetrazines; Temozolomide; Antineoplastic agents

The outstanding antineoplastic activity exhibited by two imidazo-tetrazinone derivatives, mitozolomide (**1**) and temozolomide (**2**), has attracted remarkable attention on azolo-tetrazine systems. Mitozolomide synthesized in 1980 [1] is the first azolo-tetrazine derivative to show excellent antineoplastic effects in a wide range of murine and xenograft tumors [2], but in the phase II clinical trials, the recommended dose was too toxic and a deep platelet damage (thrombocytopenia) due to cross-linking of the two strands of the DNA compromised its clinical use [3]. The 3-methyl congener, temozolomide. showed to be less potent and less toxic than mitozolomide and is used currently in therapy due to good results in patients affected by malignant melanoma, mycosis fungoides and brain tumors [4]. Most syntheses of azolo-tetrazinones have involved a slow reaction of the corresponding diazoazoles with alkyl- or arylisocyanates at room temperature (r.t.), in the dark, in an inert organic solvent [5]. Pyrrolo[2,1-*d*][1,2,3,4,5]tetrazine ring system (**3**), having, the deaza skeleton of temozolomide, has been prepared by this route [6]. The key intermediates for this synthesis are the 2-diazopyrroles which were obtained only recently in preparative yields [7].



1 R=CH_2CH_2Cl
2 R=Me

3 **4**

Several pyrrolo-tetrazinone derivatives showed antiproliferative activity with GI_{50} in the range 10^{-6}–10^{-7} M and two of them were chosen for the in vivo hollow fiber assays. Considering the potent activity showed by these compounds and in the attempt to avoid the handling of the rather unstable 2-diazopyrroles in the preparation of a wider panel of derivatives to explore SAR, we tried to obtain the pyrrolo-tetrazine system by an alternative synthesis, i.e. carbamoylation of the 2-aminopyrroles and subsequent diazotization of the corresponding 1-carbamoyl-2-amino derivatives (**4**) leading, by intramolecular cyclization, to **3**. The first step of this route was achieved in excellent yields (88–97%), whereas the title ring system **3** could not be isolated by diazotization of the intermediates **4** under several reaction conditions. An evaluation of the biological activity, in this series of pyrrolo tetrazinones indicates that the mode of action of these compounds differs from that of temozolomide. For instance, the derivative bearing a methyl group and a carboxyamide group in the **3** and **8** positions, respectively, as in temozolomide, is two orders of magnitude less potent

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than the most active derivative in the series which bears a phenyl group and a cyano group, respectively in the same positions. This evaluation is confirmed by a compare analysis of the most active pyrrolo-tetrazinone against temozolomide, which showed a very poor Pearson correlation coefficient (PCC), 0.149, at GI_{50} level and even lower, 0.045, at TGI. In fact there is practically no correlation strongly suggesting a different mechanism of action. The same compound does not compare with standard agents at the GI_{50} and TGI levels since the closest match is the *S*-trityl-*L*-cysteine, an aminoacyl-tRNA synthetase inhibitor (PCC = 0.500) at GI_{50} level and vinblastine sulfate, an antitubulin agent (PCC = 0.536) at TGI level. These data suggest that the antiproliferative activity of pyrrolo[2,1-*d*][1,2,3,4,5]tetrazines is mechanistically unrelated to that of any known drug and make this class of compounds worthy of great attention. It is our intention to undertake studies directed to elucidate the biochemical mechanism of action.

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