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Biological evaluation of Pyrrolo[3,4-*h*]quinazolines

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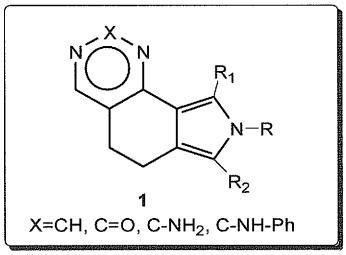
The quinazoline nucleus is the scaffold of many antitumor drugs mainly acting as inhibitors of tyrosine kinase receptors (TKR) that are overexpressed in a number of cancers. Leading examples of quinazoline based inhibitors are the clinically approved anticancer agent Iressa (ZD1839)[1] and Tarceva (OSI 774/CP358,774) which received marketing approval by the US FDA for metastatic non-small-cell lung cancer.[2,3] In the light of the potent antitumor activity of the above mentioned quinazoline derivatives, we synthetized a new pyrrolo fused heterocycle containing the quinazoline moiety, namely the pyrrolo[3,4-*h*]quinazoline **1**.



1

X=CH, C=O, C-NH_2, C-NH-Ph

Several derivatives of the title ring system were prepared with a good substitution pattern by annelation of the pyrimidine moiety on the isoindole system. Cytotoxicity was determined by MTT test after 72 h of incubation with compounds in 5 human cell lines: K-562 (chronic myeloid leukaemia), Jurkat (T-cell leukaemia), LoVo (colon carcinoma), MCF-7 (breast carcinoma) and A-431 (vulvar squamous cell carcinoma EGFR-overexpressing).[4] Some compounds resulted non-cytotoxic at the employed concentrations; others were cytotoxic with GI_{50} in the 1-19 μM range. Flow cytometry tests (Annexin V/PI test and cell cycle analysis)[5,6] were performed to investigate the kind of cellular death (necrosis or apoptosis) induced by pyrroloquinazolines and the tested compounds demonstrated to induce cell death by apoptosis. Further analysis were carried out to check the involvement of mitochondria and lysosomes in the apoptotic process.[7,8] Moreover, the human cell line A-431, which overexpresses EGFR, was used to investigate whether these compounds could block EGF receptor signaling. However no reduction in its phosphorylation was observed, so EGFR is not the target. Cell-cycle analysis were performed on Jurkat cells incubated for 24 h with the most active compounds and a concentration-dependent increase of cells in G2/M phase with the onset of a subG1 peak was observed. Further studies indicated that compounds **1** were able to induce deep changes in the microtubule network so they could act as antimitotic drugs and further investigations will be performed to confirm their mechanism of action.

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