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Original article

Aza-isoindolo and isoindolo-azaquinoxaline derivatives with antiproliferative activity

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ABSTRACT

Three new ring systems, pyrido[2′,3′:3,4]pyrrolo[1,2-*a*]quinoxalines, pyrido[3′,2′:3,4]pyrrolo[1,2-*a*]quinoxalines and pyrido[2′,3′:5,6]pyrazino[2,1-*a*]isoindoles, were synthesized through an aza-substitution on the already active isoindolo-quinoxaline system and in particular in the position 7 or 4 of the isoindole moiety and in position 5 of the quinoxaline portion. All new compounds were screened by the National Cancer Institute (Bethesda, MD) against a panel of 60 human tumor cell lines. Biological results of the most active derivatives, with pGI_{50} values between 7.09 and 7.27, confirmed the importance of the presence of methoxy substituents for biological activity. The anti-proliferative effect of selected quinoxalines was associated with apoptosis of the cells and arrest in G2/M phase of the cell cycle. DNA binding properties of the compounds was also assessed to investigate the possible mechanism of action.

1. Introduction

Quinoxaline and its derivatives constitute an important class of benzo-fused heterocycles which exhibit remarkable and variegate biological activities. In fact both suitable decoration with selected substituents and annelation with other heteocyclic systems have contributed to provide derivatives with a high degree of diversity allowing different biological activities such as antimalarial [1], antiinflammatory [2], anti-Alzheimer's disease [3]. Several quinoxalines also exhibit antineoplastic activity [4–6]. For instance, 6-triazolyl substituted quinoxaline derivatives of type **1** inhibited activin receptor-like kinase 5 (ALK5) with IC_{50} values in the low micromolar range [7] (Chart 1). Substituted (phenoxymethyl)quinoxalinones of type **2** demonstrated excellent antagonism of P-glycoprotein and multidrug resistant protein (MRP1) in drug-resistant cell lines [8]. Also quinoxalines annelated to heterocyclic moieties showed interesting antitumor activity.

Thus, imidazo[1,2-*a*]quinoxaline derivatives of type **3** exhibited *in vitro* and *in vivo* cytotoxic activity against A375 and M4Be human melanoma cell lines superior to that of the reference drugs imiquimod and fotemustine [9]. Quinoxaline ring condensed with a pyridine moiety produced suitably substituted derivatives of the ring system pyrido[3,4-*b*]quinoxaline **4** and the corresponding benzo-fused benzo[*f*]pyrido[4,3-*b*]quinoxaline possessing topoisomerases inhibition [10]. Fusion with an indole ring gave highly active derivatives of the ring system 6*H*-indolo[2,3-*b*]quinoxaline **5** which have shown good binding affinity to DNA as evident from high thermal stability of compound-DNA complexes. They also exhibited significant MDR modulating activity [11]. In the framework of our researches on polycyclic nitrogen systems, we obtained several ring systems bearing the pyrrole [12–24], the benzo-fused indole [25–34], or indazole [35,36], moieties with chemotherapeutic or photochemotherapeutic activity. More recently, we extended our interest to the isoindole system with the aim to study the reactivity of this relatively recent heterocycle and search for novel antitumor compounds [37–39]. Derivatives of the tetracyclic new ring system isoindolo[2,1-*a*]quinoxaline (IIQ) showed potent antiproliferative activity with GI_{50} values in the low micromolar –

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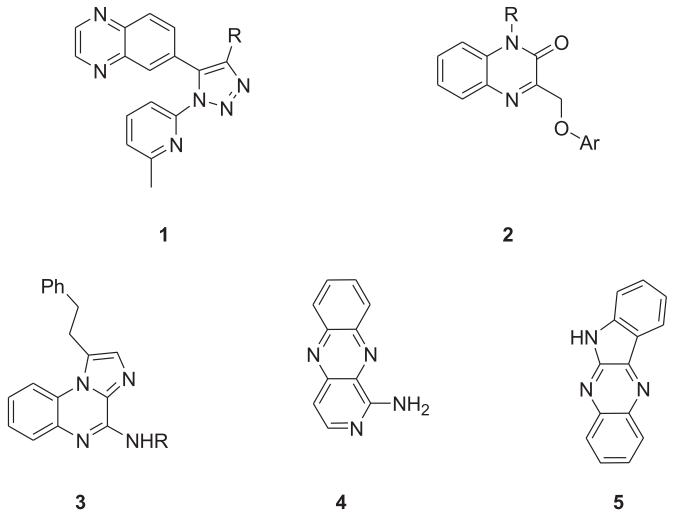


Chart 1.

nanomolar range against the NCI panel of about 60 human tumor cell lines and against vinblastine and doxorubicin resistant cell lines [40]. These compounds arrested the cell cycle in the G2/M phase with concomitant apoptosis of the cells, mitochondrial depolarization, generation of reactive oxygen species, and activation of caspase-3 and caspase-9. Moreover, IIQs induced a clear increase in the mitotic index, inhibited microtubule assembly *in vitro*, and also acted as a topoisomerase I inhibitors. Successively, more soluble derivatives isoindolo[2,1-*a*]quinoxalin-6-imines and their corresponding acetate salts were synthesized and the antiproliferative activity had a remarkable increase, in fact the most potent compound showed GI_{50} values at a nanomolar level on 90% of the tested cell lines. It was demonstrated that, among novel derivatives, methoxy-substituted ones were able to induce impairment of cell cycle progression and apototsis as a result of their interference with different relevant biological targets. The compounds affected tubulin polymerization, induced perturbation in telomere architecture, according to their G quadruplex-stabilizing activity, and impaired DNA topoisomerase I functions [41].

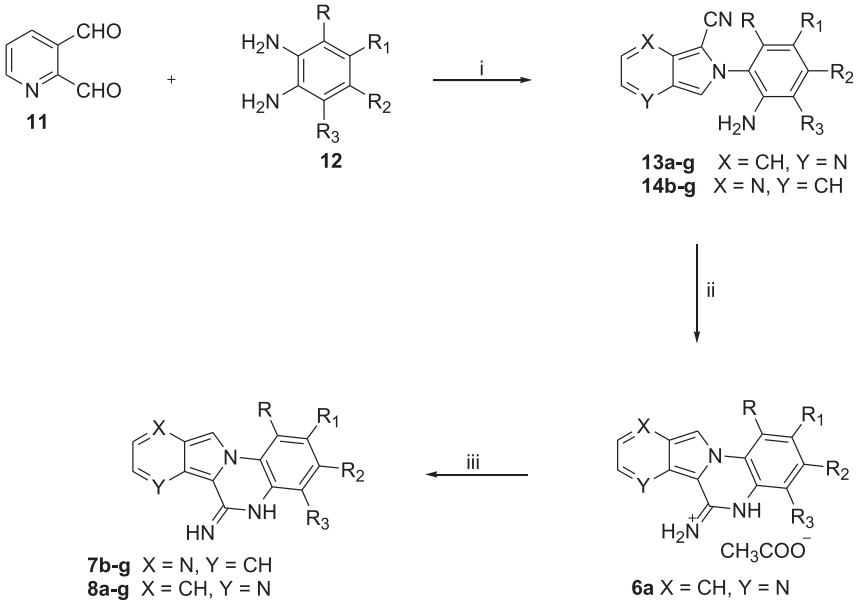
In this paper we report the synthesis of derivatives of three new ring systems deriving from an aza-substitution on the already active isoindolo-quinoxaline system and in particular in the position 7 or 4 of the isoindole moiety and in position 5 of the quinoxaline portion which gives rise to pyrido[2′,3′:3,4]pyrrolo[1,2-*a*] quinoxalines **6a**, **8a**–**g**, pyrido[3′,2′:3,4]pyrrolo[1,2-*a*]quinoxalines **7b**–**g** and pyrido[2′,3′:5,6]pyrazino[2,1-*a*]isoindoles **9a**–**c** and **10a**–**c** respectively. The novel compounds were examined in their cytotoxic action against the NCI panel of 60 human cancer cell lines. To investigate the mechanism of the anti-proliferative effect, apoptotic activity of selected derivatives were assessed on human colorectal cancer cell line HCT116. Studies on DNA binding properties suggest that their cytotoxic action is not due to interference with nucleic acids.

2. Results and discussion

2.1. Chemistry

Pyrido[2′,3′:3,4]pyrrolo[1,2-*a*]quinoxalines **6a**, **8a**–**g** and pyrido [3′,2′:3,4]pyrrolo[1,2-a]quinoxalines **7b**–**g** were respectively obtained by using azaisoindole carbonitriles **13b**–**g** and **14a**–**g** as key intermediates (Scheme 1). These latter compounds were prepared (40–80%) by a Strecker-type reaction between the substituted 1,2-phenylendiamines **12a**–**g** and pyridine-2,3-dicarbaldehyde **11** in water and in the presence of potassium cyanide and sodium hydrogen sulfite. The commercially unavailable pyridine **11** and 1,2-phenylenediamines **12c**–**e** were synthesized as previously reported [42–45]. Due to asymmetry of the pyridine aldehyde **11** two different classes of carbonitriles of type **13** and **14** were isolated with a nitrogen atom in position 7 or 4 of the isoindole moiety respectively.

All the synthesized aza-carbonitriles **13b**–**g** and **14a**–**g**, at reflux in glacial acetic acid, gave the desired quinoxalines **6a**, **7b**–**g** and **8a**–**g** (Table 1). In particular, only one acetate **6a** was immediately obtained on cooling crystallized in a pure form (60%) while the neutralization, at closely controlled temperature of −5 °C, of the



Scheme 1. *Reagents and conditions*: (i) (a) $NaHSO_3/H_2O$, rt or steam bath, 1 h; (b) KCN/H_2O, rt or steam bath, 5 h, 40–80%; (ii) AcOH, reflux, 0.5–6 h, 60%; (iii) $NaHCO_3/H_2O$, −5 °C, 40–99%.