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The discovery of new cytotoxic pyrazolopyridine derivatives

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ABSTRACT

A number of new 3,7-disubstituted pyrazolo[3,4-*c*]pyridines have been designed and synthesized from suitable 2-aminopyridines. The antiproliferative activity of the derivatives was determined against the pancreatic MIA PaCa-2 and ovarian SCOV3 cancer cell-lines. IC_{50} values of the most promising analogue **46** lie in the submicromolar or low micromolar range. Furthermore, compound **46** shows similar inhibitory activities against DU145, A2058 and PC-3 cancer cells, blocks the cell cycle at the G_0/G_1 phase and induce apoptosis, as determined by the appearance of apoptotic nuclei.

In addition to their contribution in encoding of genetic information, the naturally occurring purines adenosine and guanosine and their nucleotide derivatives play crucial roles in a number of biological processes, mainly involved in cell metabolism and cell proliferation.[1] Many cellular proteins contain a purine recognition pocket for their interaction with their corresponding energy intermediates, substrates, allosteric modulators or cofactors, therefore, numerous purine derivatives have been designed and developed to selectively inhibit these enzymes and receptors.[2] Depending on their substitution pattern and the wide variety of the substituents, these compounds were found to exhibit a broad range of biological and pharmaceutical properties.[3–6] Besides serving as privileged medicinal chemistry scaffolds, these derivatives also inspired parallel development of alternative heterocyclic isosteres, in the attempt to explore the therapeutic potential or improve the physicochemical and pharmacokinetic properties of the corresponding purine derivatives.[7–10] However, all these compounds must closely mimic the overall shape of the endogenous natural products and retain certain hydrogen bond donor/acceptor binding motifs, in order to maintain the ability to substitute for them in various biological processes. Changing the location of a nitrogen atom from position 9- to position 8- in the purine ring leads to pyrazolopyrimidines, which were initially reported as adenosine receptor antagonists, and were further found to exert potent anti-inflammatory,[11] anti-viral[12] and anticancer properties.[13,14] The closely related pyrazolopyridine core, which can be considered as the bioisosteric deazapyrazolopyrimidine scaffold, can also serve as pharmacophore for the discovery of biologically interesting compounds.[15,16] The anticancer potential of several pyrazolo[3,4-*d*]pyridines has been already studied,[13] as well as their investigated molecular mechanisms, such as inhibition of EGFR,[17] IGF-1R[18] and dual Src/Abl.[19]

In the course of our involvement in the synthesis and biological activity evaluation of pyrazolo[3,4-*c*]pyridine derivatives[20–22] we have initiated a project towards the study of the impact of certain substitution patterns on this interesting scaffold. We thus present herein the synthesis of some new 3,7-disubstituted pyrazolo[3,4-*c*]pyridines and their antiproliferative activity against cancer cell lines.

The new compounds were prepared using as starting materials the commercial aminopicoline **1**, as well as the substituted aminopyridine **6** (Scheme 1). The later compound has not been

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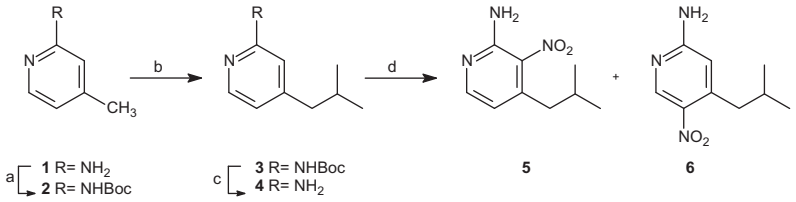
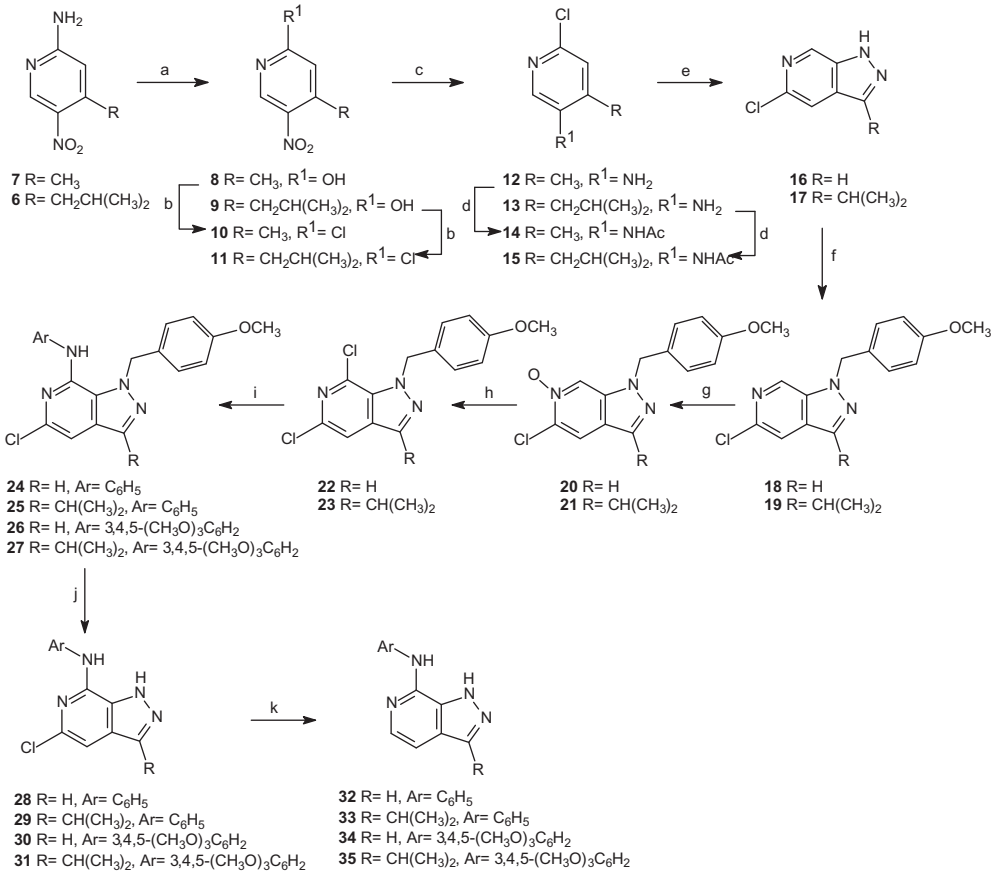


Scheme 1. Reagents and conditions. (a) Boc_2O, *t*-BuOH, rt; (b) (i) *n*-BuLi (2.5 equiv), THF dry, −80 °C, then rt, (ii) isopropylbromide, −80 °C, then rt; (c) HCl (5 N), EtOH, rt; (d) HNO_3, H_2SO_4, 65 °C.

previously reported and was synthesized in four steps, according to the reactions depicted in Scheme 1. Thus, the picoline **1** was first protected through conversion to the corresponding carbamate **2**, which was lithiated using 2.5 equiv. of *n*-butyllithium, in THF at −80 °C and the resulting 4-methylene anion attacked isopropylbromide to provide the pyridine **3**. Compound **3** was then deprotected in acidic media and the resulting amine **4** was nitrated to give both nitroderivatives **5** and **6**, which were chromatographically separated and identified.

The synthesis of the target 7-substituted pyrazolo[3,4-*c*]pyridines as well as the corresponding 3-isopropyl analogues is depicted in Scheme 2. We used either 2-amino-5-nitro-4-picoline (**7**), which was prepared from the picoline **1** following a previously reported methodology[23], or the homologous picoline **6**. These aminopyridines were subjected to diazotization and the resulting pyridinones were treated with phosphorous oxychloride to result in the chlorides **10**[24] and **11**. The 5-nitro group of the afore mentioned chlorides was reduced, using tin (II) chloride in HCl and the amines thus prepared, **12** and **13**, were acetylated to provide the acetamides **14** and **15**. Each acetamide was then treated with isoamyl nitrite in benzene at reflux in the presence of acetic anhydride and potassium acetate,[25,26] to result upon rearrangement of the intermediate *N*-nitroso compound in 1-acetyl-5-chloropyrazolo [3,4-*c*]pyridine or 1-acetyl-5-chloro-3-isopropylpyrazolo[3,4-*c*] pyridine. The acetyl group was cleaved upon treatment with methanolic ammonia to result in derivatives **16**[26] and **17**. The pyrazole NH of these derivatives was protected using the 4-methoxybenzyl group, upon reaction with 4-methoxybenzylchloride in the presence of NaH. In the case of **17**, we have obtained selectively the *N*1-substituted isomer, due to the presence of the bulky 3-isopropyl group. On the contrary, concerning compound **16**, we isolated both *N*1 and *N*2 regio-isomers which were separated and identified using NOE spectroscopic data.[27]

The protected derivatives **18** and **19** were converted to the corresponding *N*-oxides **20**[27] and **21**, using *m*-CPBA as oxidizing agent. The rearrangement of the *N*-oxides in the presence of phosphorous



Scheme 2. Reagents and conditions. (a) $NaNO_2$, H_2SO_4, H_2O; (b) $POCl_3$, 100 °C; (c) $SnCl_2{\cdot}2H_2O$, HCl(c.), 55 °C for **12** or rt for **13**; (d) Ac_2O, CH_2Cl_2, rt; (e) (i) AcOK, Ac_2O, isoamyl nitrite, benzene, reflux, (ii) NH_3(g.), MeOH, rt; (f) (i) NaH, DMF, rt, (ii) 4-methoxybenzyl chloride, DMF, rt; (g) *m*-CPBA, $CHCl_3$, rt; (h) $POCl_3$, THF, rt; (i) NaH, aniline (for **24**, **25**) or 3,4,5-trimethoxyaniline (for **26**, **27**), DMF, 100 °C; (j) trifluoroacetic acid, rt; (k) Pd/C, H_2, AcOK, EtOH, 50 psi, rt.