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Original article

Novel 5,7-disubstituted 6-amino-5H-pyrrolo [3,2-b]pyrazine-2,3-dicarbonitriles, the promising protein kinase inhibitors with antiproliferative activity

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Abstract

New derivatives of pyrrolo[2,3-b]pyrazine were synthesized and tested on a panel of cultured human tumor cell lines. It was found that 6-amino-5-(3-chlorophenylamino)-7-(1-methyl-1H-benzo[d]imidazol-2-yl)-5H-pyrrolo[3,2-b]pyrazine-2,3-dicarbonitrile (**4j**) exhibited a significant antiproliferative activity: GI50 for cell lines RXF 393 (renal cancer) and BT-549 (breast cancer) were 14 and 82 nM, respectively. To identify possible molecular targets, docking of the most active compounds into the active sites of cyclin-dependent kinases was performed. Molecular modeling of the inhibitor–enzyme complexes showed the differences in the binding poses of new pyrrolo[2,3-b]pyrazine derivatives in the kinase ATP-binding site compared with known pyrrolo[2,3-b]pyrazine inhibitors called aloisines. The patterns of drug kinase interactions correlated well with antiproliferative activities of novel derivatives. Key interactions and binding mode of docked compounds are discussed.

Keywords: Antiproliferative agents; Cytostatic agents; Pyrrolo[2,3-b]pyrazine; Kinase inhibitor; Molecular modeling; Docking

1. Introduction

The derivatives of pyrrolo[2,3-b]pyrazine are known to be biologically active. In addition to having antibronchospastic effect [1] and the ability to inhibit the activity of p38 MAP kinase [2], the compounds of this class can also inhibit cyclin-dependent kinases (CDKs) and glycogen synthase kinase-3 (GSK-3), thereby exerting an antiproliferative effect [3–6]. The most efficient pyrrolo[2,3-b]pyrazine derivatives are aloisines. Fig. 1 shows the structures of aloisines A and B.

Aloisines bind to the kinase ATP-binding pocket as competitive inhibitors [4,6]. The structures of the active sites and interaction patterns of the kinases with aloisine A and B (aloisine B-CDK2 and aloisine A-CDK5 (1ung.pdb) complexes; [4]) have been investigated by the X-ray method. The authors found that the key interactions were the two hydrogen bonds with the backbone nitrogen and oxygen atoms of Leu 83 (CDK2) and Cys83 (CDK5) formed by the nitrogen atom in position 4 and the hydrogen atom adjacent to N(5) of the pyrrolo[2,3-b]pyrazine cycle. The third hydrogen bond was formed between the nitrogen atom N(1) and ε-amino group of Lys33 (aloisine B/CDK2) [4] and the nitrogen N(1) of aloisine A was engaged in an hydrogen bonding network involving the side chains of Lys33, Glu51, Asn144, and two water molecules (CDK5) [6]. Structure–activity relationship (SAR) studies of 50 aloisine derivatives with different substituents in the positions **2**, **3**, **7a**–**e** (Fig. 1) were performed [4]. The replacement by carbon atom or an alkylation of any of the nitrogen atoms that formed the essential hydrogen bonds dramatically de-

Abbreviations: CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor kinase; GI_{50}, growth inhibition; GSK-3, glycogen synthase kinase-3; LC_{50}, lethal concentration; LC/MS, liquid chromatography/mass spectrometry; MAP, mitogen activated protein kinase; PG, percentage growth; RMSD, root mean square deviation; SAR, structure–activity relationship; SRB, sulforhodamine B; TGI, total growth inhibition; t_R, retention time.

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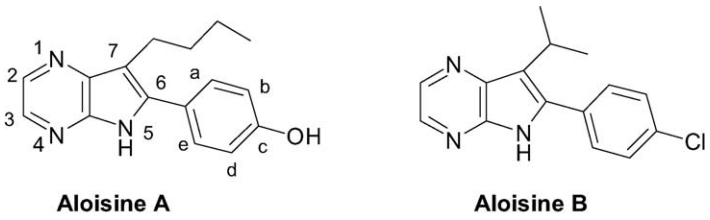


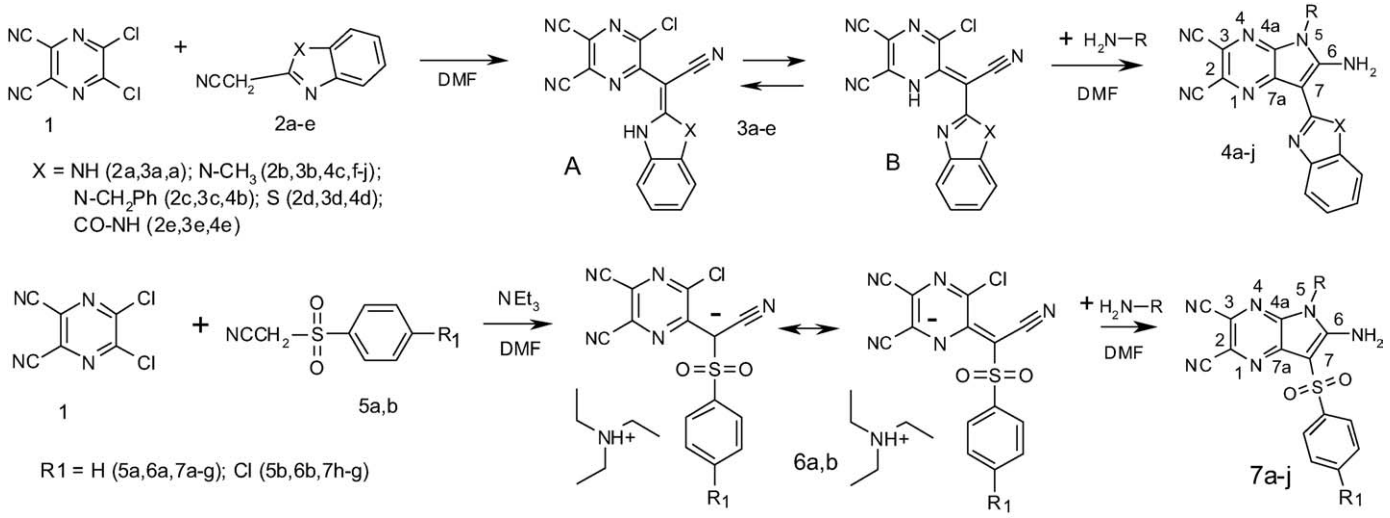
Fig. 1. Aloisine A and B, the inhibitors of CDKs and GSK-3.

creased the inhibitory activity. Introducing one, two, three methoxy groups at the positions **a**–**d** of the phenyl ring or its replacement by five or six membered heterocycles and the presence of any substituents at the position 2,3 of pyrrolo[2,3-b] pyrazine cycle decreased the inhibitory activity. In contrast, the presence of alkyl substituent at the position 7 and chlorine atom or hydroxy group at the **c**-position of the phenyl ring increased the anti-enzymatic effect. These facts correlated well with X-ray data on interaction of these groups with the side chains and hydrophobic pockets of the binding sites [4,6].

2. Chemistry

In this work, 20 new pyrrolo[2,3-b]pyrazine derivatives were synthesized, namely, 5,7-disubstituted 6-aminopyrrolo [2,3-b]pyrazine-2,3-dicarbonitriles **4a**–**j**, **7a**–**j** (Scheme 1). A two-stage nucleophilic substitution of chlorine atoms in 5,6-dichloropyrazine-2,3-dicarbonitrile **1** with C- and N-nucleophiles was used for building the pyrrolo[2,3-b]pyrazine cycle (Scheme 1). α-Azaheteroarylacetonitriles **2a**–**e** and 2-(phenylsulfonyl)acetonitriles **5a**, **b** were used in the first step as C-nucleophiles (Scheme 1) to produce **3a**–**e** and **6a**, **b**. These products can exist in two tautomeric forms, A and B [7]. The next step was the nucleophilic substitution of the second chlorine atom by N-nucleophiles, primary amines and 3-chlorophenylhydrazine (for compound **4j**), followed by the addition of the secondary amine to nitrile group and formation of the pyrrolo [2,3-b]pyrazine cycle. Compounds **4a**–**e** were synthesized as described earlier in [7], and the derivatives **4f**–**j** were obtained. Compounds **6a**, **b** were synthesized as salts presented by two resonance structures, where the negative charge is delocalized between the carbon atom of the acetonitrile moiety and the nitrogen atom of the pyrazine ring. The salt formation can be explained by high C–H acidity of the carbon atom that has three electron-withdrawing groups. Thus, a strong C–H acid and hydrochloric acid were formed in this reaction. These acids competitively protonated triethylamine (TEA) used as a base. Due to this reason, the highest yields were obtained with 2 equiv. of TEA. In the previous study we investigated the formation of a salt product in the reaction of 5,6-dichloropyrazine-2,3-dicarbonitrile with malononitrile and TEA as a base, and found that the C–H acid with pK_a = 0.84 was obtained [8]. The interaction of compounds **6a**, **b** with primary amines formed pyrrolo[2,3-b]pyrazine **7a**–**j**.

^{1}H NMR spectra of compounds **4a**–**j**, **7a**–**j** showed a singlet of the amino group protons at 8.21–9.85 ppm (s, 2H, NH_2) that disappeared after the addition of D_2O. Singlets of the labile protons of compounds **3a**–**e** at 12.58–12.76 ppm were absent in the spectra of compounds **4a**–**j**, and multiplet signals of H–N^+(Et)$_3$ of compounds **6a**, **b** at 10.74–11.20 ppm were absent in the spectra of products **7a**–**j**. In compounds **3a**–**e**, **6a**, **b** the characteristic strong band of the conjugated nitrile group of the acetonitrile moiety was observed in IR spectra (2195–2185 cm^{-1}). This band was not present in the IR spectra of products **4a**–**j**, **7a**–**j**. Low intensity bands of pyrazine nitrile bonds were observed in the 2227–2210 cm^{-1} range.



Scheme 1.