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Design, synthesis, structure–activity relationship and kinase inhibitory activity of substituted 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones

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ABSTRACT

A new series of 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones having variable substitutions at N5 and C6 positions has been synthesized and characterized. The synthesized compounds were tested for cytotoxicity against K562 and MCF-7 cancer cell lines and for inhibition of protein kinases CDK1/cyclin B, CDK2/cyclin E and Abl. Compounds **5f** and **5h** killed both K562 and MCF-7 cell lines with IC_{50} values 8.2, 9.6 μM and 15.3, 10.8 μM, respectively. In addition, **5f** and **5h** showed antiproliferative effect through arrest in G2/M phase on cell cycle of K562 cancer cell line in a dose-dependant manner. To confirm the mechanism of cell death, activity of caspase-3/7 was measured. Moreover, kinase selectivity profiling of the most potent compound **5f** revealed several other sensitive targets, including RSK1 and RIPK2, TrkA and VEGFR. The results provide a starting point for optimization in order to increase their potency against kinases and cancer cell lines.

Naturally occurring purines play vital roles in many biological processes. Therefore, numerous libraries of synthetic purine derivatives have been prepared with the aim to find drugs for diseases caused by aberrant purine-binding proteins.[1] Besides various purines, many related heterocyclic systems have been prepared to enlarge the chemical space for drug discovery, including several pyrazolopyrimidine types. Biological activity of pyrazolopyrimidines is dictated both by the type of condensation between the pyrimidine and the pyrazole rings and by the nature of their substitutions. Pyrazolo[3,4-*d*]pyrimidines have attracted especial interest for at least two reasons; they are highly amenable to the chemical manipulations and display strong anticancer activities.[2] Many of these compounds target protein kinases—enzymes that are involved in majority of cellular processes—starting with extracellular signaling by membrane receptors and its transduction down to the regulation of gene expression, metabolism and proliferation. Today, specific inhibitors for many of these kinases are known (Fig. 1), including those of receptor tyrosine kinases,[3–6] non-receptor tyrosine kinases Src,[7,8] HCK,[9] Abl,[10,11] ACK1[12] or serin/threonin kinases p38α/MAPK,[13] GSK3[14] and CDKs.[15–17] Comprehensive recent review on this topic has been published recently.[18]

Many researches have been focused primarily on CDK inhibitors with the purine scaffold that yielded roscovitine, a compound undergoing clinical trials.[19–21] The success of roscovitine has prompted and guided parallel advances in the preparation of CDK inhibitors built on related heterocyclic systems.[22] The most active bioisosteres have either pyrazolo[1,5-*a*]pyrimidine or pyrazolo[4,3-*d*]pyrimidine core.[23,24] Interestingly, pyrazolo[3,4-*d*]pyrimidines with substitution analogous to that of roscovitine did not show reasonable kinase inhibitory activity.[15] This class of compounds lacks the typical substitution pattern and thus cannot bind to CDK2 in the same manner like roscovitine.[22] However, when 1-isopropyl moiety (analogous to 9-isopropyl in roscovitine) was omitted from the structure, the compounds regained the affinity to CDK, but the binding was in a reversed manner.[15] The pyrazolo[3,4-*d*]pyrimidines substitution pattern was further optimized and resulting compounds proved to be potent CDK inhibitors,[16] although these cannot be considered roscovitine bioisosteres any longer due to their reversed binding within CDK2. Yet another reported group of kinase inhibitors based on 1,6-disubstituted pyrazolo[3,4-*d*]pyrimidines (see **15a** in Fig. 1) lacks 4-oxo function and the compounds bind to aurora A kinase (and possibly also to CDK2) somehow shifted, using endocyclic N5 as an acceptor of H bond, and exocyclic NH at 6 as a donor.[25] Similar binding mode is used by 1,3,6-trisubstituted pyrazolo[3,4-*d*]pyrimidine inhibitors of ACK.[26]

The aim of the present study was to prepare 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones having variable substitutions not only at C6, but also at previously unexplored N5

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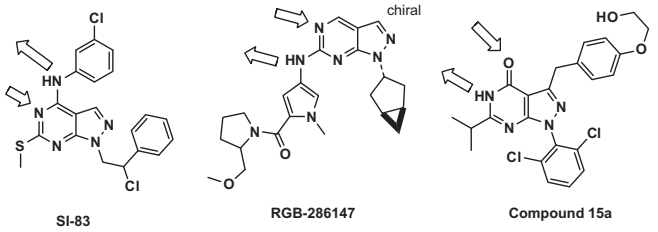


Figure 1. Pyrazolo[3,4-*d*]pyrimidine inhibitors of Src (**SI-83**), CDKs (**RGB-286147**) and aurora A (compound **15a**) kinases. Arrows indicate H-bond donors and acceptors interacting with the protein backbone.

position, and to describe relationships between these modifications and their impact on CDK inhibition.

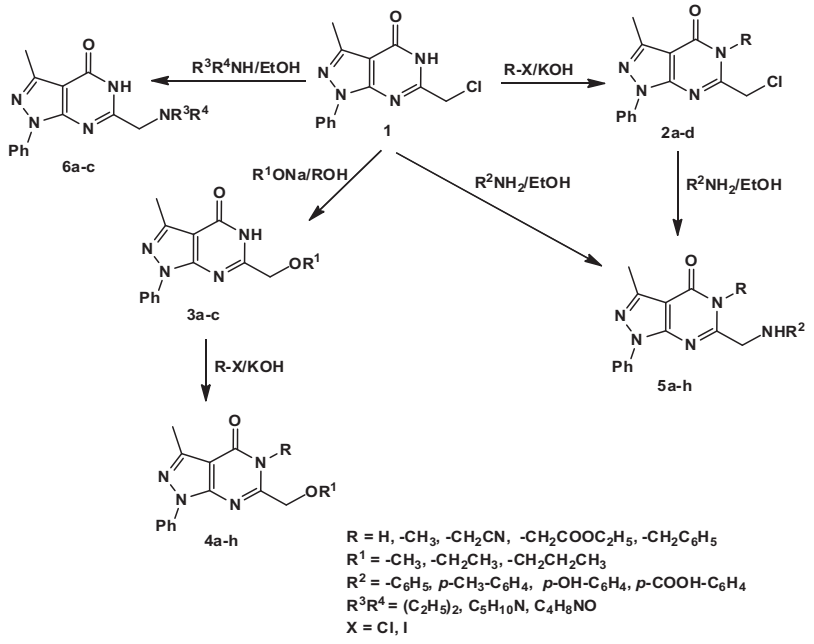
The preparation of pyrazolo[3,4-*d*]pyrimidine derivatives **2a**–**d**, **4a**–**h** and **5e**–**h** bearing different substituents at N5 and C6 positions is illustrated in Scheme 1. Synthesis and characterization of compounds without a substitution at N5 position (**1**, **3a**–**c**, **5a**–**d** and **6a**–**c**) was described in previously published work.[27]

Alkylation at N5 position was performed by heating **1** with alkyl or aryl halide equivalents (methyl iodide, chloroacetonitrile, ethyl chloroacetate and benzyl chloride) in refluxing absolute ethanol containing potassium hydroxide to give **2a**–**d**. Compound **1** was converted to the key intermediates **3a**–**c** by heating under reflux with an alkali metal alkoxide in methanol, ethanol or propanol by a route which was reported earlier.[27] The incorporation of diverse groups in N5 position along with methoxy or ethoxy methyl group at the C6 position (compounds **4a**–**h**) was performed using the same alkylation conditions adopted for compounds **2a**–**d**. Nucleophilic substitution reaction with aniline or *p*-hydroxy aniline on the chlorine atom in 2**a&b** containing methyl or cyanomethyl group at N5 afforded new compounds **5e**–**h**. While, secondary amines namely diethyl amine, piperidine and morpholine were previously used to synthesized compounds **6a**–**c**. IR, ^{1}H NMR, ^{13}C NMR and mass spectral data of all the newly synthesized compounds were recorded and found to be in agreement with the proposed structures.

All synthesized compounds, including those derivatives described recently,[27] were tested for cytotoxicity against cancer cell lines MCF7 and K562 and for inhibition of protein kinases CDK1/cyclin B, CDK2/cyclin E and Abl. The resulting data presented in Table 1 clearly show that some compounds have single-digit micromolar IC_{50} values towards CDKs; complete lack of sensitivity of Abl demonstrates some degree of selectivity. Unexpectedly, majority of compounds did not display cytotoxicity in cancer cell lines despite relatively reasonable potency against CDK1 and CDK2, probably due to limited solubility in a culture medium. The two exceptions, compounds **5f** and **5h**, killed both cell lines with IC_{50} values in a micromolar range. Interestingly, these two compounds were not the most potent CDK inhibitors from the series.

The structure–activity relationships of 1*H*-1-phenylpyrazolo [3,4-*d*]pyrimidin-4-ones substituted in positions 5 and 6 have been studied. 3-Methyl and 1-phenyl side chains on this skeleton have been previously identified as acceptable in terms of CDK inhibition[16] and due to the synthetic strategy; these substitutions were kept unchanged in this study. The assays identified 6-phenylaminomethyl derivative **5a** as the most potent CDK2 inhibitor from the series. However, further decoration of the phenyl ring in *para* position decreased slightly the activity of **5b**–**d**. Compounds without the aromatic substitution in position 6 lost the activity completely; we observed this for ethers **3a**–**c** and derivatives having side chains with tertiary amines **6a**–**c**.

This observation is in line with that one published by Markwalder et al.,[16] who studied CDK inhibitory activity of 1-aryl-4,5-dihydro-1(*H*)-pyrazolo[3,4-*d*]pyrimidin-4-ones and found that position 6 can accept variety of substitutions. Although CDK4/D1 kinase was largely insensitive to changes in position 6, CDK2/E displayed strong sensitivity to 6-benzyl derivatives over other substitutions. This also correlates with these results, because no activity has been observed for compounds with non-aromatic substitutions (compounds **6a**–**c**). However, further modification of the benzyl ring lead to drop in activity of **5b**–**d** versus **5a**. This was reasoned for one-atom longer substitution in position 6 (phenylaminomethyl vs benzyl) is suboptimal for binding to CDK2.



Scheme 1. Synthesis of N5, C6-disubstituted pyrazolo[3,4-*d*]pyrimidine derivatives.