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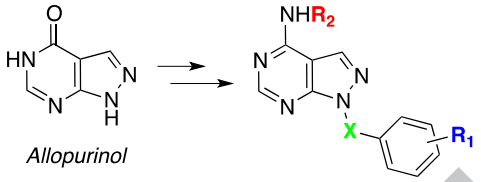

Allopurinol

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An alternative synthetic approach for the synthesis of biologically relevant 1,4-disubstituted pyrazolo[3,4-*d*]pyrimidines

Marco Radi,[a,b] Vincenzo Bernardo,[a] Giulia Vignaroli,[a] Annalaura Brai,[a] Mariangela Biava,[c] Silvia Schenone[d] and Maurizio Botta[a,e]*

[a] *Dipartimento di Bioteconologie, Chimica e Farmacia, Università degli Studi di Siena Via Alcide de Gasperi, 2, 53100 Siena, Italy*
[b] *Dipartimento di Farmacia, Università degli Studi di Parma, Viale delle Scienze, 27/A, 43124 Parma, Italy*
[c] *Dipartimento di Chimica e Tecnologie del Farmaco, Università degli Studi di Roma "La Sapienza", Piazzale Aldo Moro 5, 00185-Roma, Italy*
[d] *Dipartimento di Farmacia, Università degli Studi di Genova, Viale Benedetto XV, 3, 16132 Genova, Italy*
[e] *Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, BioLife Science Building, Suite 333, 1900 N 12th Street, Philadelphia, PA, USA*

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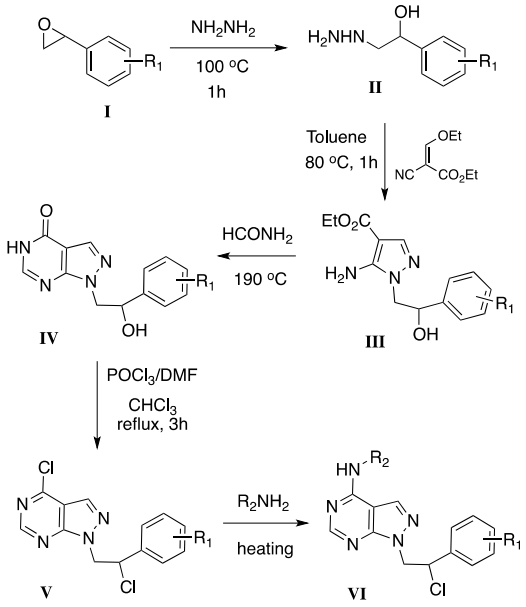
ABSTRACT

A versatile approach for the synthesis of 1,4-disubstituted pyrazolo[3,4-*d*]pyrimidines has been developed. Starting from commercially available allopurinol, TBAF mediated N1-functionalization and subsequent C4 nucleophilic substitution, under microwave assisted- or standard heating conditions, allowed to access to highly functionalized pyrazolo[3,4-*d*]pyrimidines of potential biological interest.

Nitrogen-containing heterocycles are widely distributed in nature and play a key role in the metabolism of all living cells. Among the many nitrogen-containing heterocycles, the pyrazolo[3,4-*d*]pyrimidine nucleus represents a very interesting and versatile scaffold for the synthesis of potential drug candidates acting on a wide range of biological targets.[1] Among their many applications, pyrazolo[3,4-*d*]pyrimidines have been used as kinase inhibitors,[2] antiviral agents,[3] adenosine antagonists,[4] glutamate modulators[5] and antitubercular agents.[6] The two most common synthetic approaches for the preparation of pyrazolo[3,4-*d*]pyrimidine derivatives build the bicyclic scaffold starting alternatively from the pyrimidine[7] and the pyrazole nucleus.[1a] However, several steps are needed in most cases to construct and functionalize the core scaffold. In the last few years, our research group has developed several pyrazolo[3,4-*d*]pyrimidines (general structure VI, Scheme 1) which proved to be promising antitumor agents active on different cancer cell lines depending on the nature and position of substituents on the heterocyclic core.[8] These compounds were synthesized starting from commercial phenyloxiranes (I) which were converted to the ethyl esters of 5-amino-1H-pyrazole-4-carboxylic acids (III) in two steps. Reaction of the latter intermediates with ethyl(ethoxymethylene)cyanoacetate followed

* Corresponding author. Tel.: +39-0577-234306; fax: +39-0577-234333; e-mail: botta.maurizio@gmail.com; maurizio.botta@unisi.it



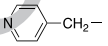
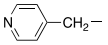
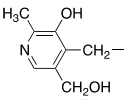
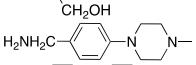
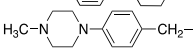
Scheme 1. Classical synthetic approach for the synthesis of antitumor pyrazolo[3,4-*d*]pyrimidines **VI**.

by treatment with an excess of formamide at 190 °C gave the N1-substituted pyrazolo-pyrimidinones (IV). The final products (VI) were then obtained after chlorination with the Vilsmeier complex ($POCl_3$:DMF, 1:1) followed by regioselective substitution of the chlorine at C4 with an excess of different amines. The main drawback of the above described synthetic approach is represented by its low versatility, especially for its application in the development of N1 substituted analogues such as VI: the chemical diversity in the N1 side chain phenyl ring is in fact determined in an early phase by the choice of the starting phenyloxiranes (I) that should be submitted to the five-step sequence described in scheme 1.

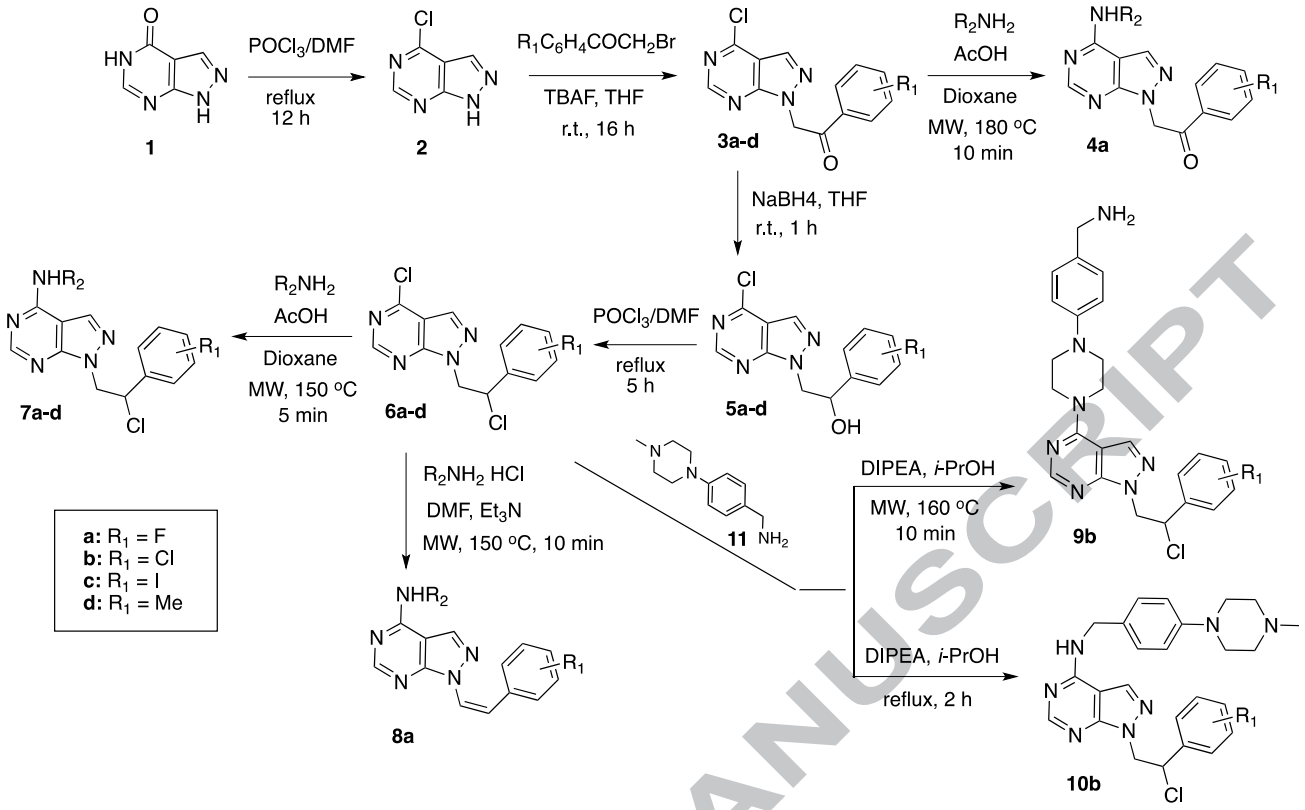
Herein we report the application of a direct N1-functionalization strategy on the preformed pyrazolo[3,4-*d*]pyrimidine scaffold as an alternative approach for the synthesis of functionalized analogues of antitumor compounds VI. A few authors reported the direct N1 substitution of the pyrazolo[3,4-*d*]pyrimidine ring, especially with short chain substituents, via alkylation[9] or Mitsunobu[10] reaction, which led to the concurrent formation of the N2 substituted derivatives. The N1/N2 substitution ratio usually depends on the base used to form the salt and is also related to the halide used in the nucleophilic substitution. Zacharie and coworkers reported the alkylation of the pyrazolo[3,4-*d*]pyrimidine scaffold with alkyl iodides in the presence of cesium carbonate or DBU in dry DMF at 0 °C leading to a mixture of N1/N2-functionalized derivatives in a 3/1 ratio.[11] Better results were later reported by Gundersen et al. which obtained low yields of the N2-functionalized side products using different alcohols under Mitsunobu conditions.[12]

On these basis, we decided to develop an alternative synthetic approach to synthesize 1,4-disubstituted pyrazolo[3,4-*d*]pyrimidine derivatives (such as VI) that could be favorably employed to increase the chemical diversity in the N1 side chain. Starting from commercial allopurinol **1**, treatment with $POCl_3$/DMF led to the C4-chloro derivative **2** that could be

Table 1. Substituted pyrazolo[3,4-*d*]pyrimidine analogues

Entry	Compound	R_1	R_2	Yield (%)
1	**4a**	4-F -	Bn	63
2	**7a**	4-F		68
3	**7b**	4-Cl		75
4	**7c**	4-I	Ph	70
5	**7d**	4-Me	Ph	60
6	**8a**	4-F		58
7	**9b**	4-Cl		67
8	**10b**	4-Cl		76

directly submitted to N1-functionalization with substituted 2-bromo 4-acetophenones to give the key intermediates **3** (Scheme 2). Unfortunately, when the N1-alkylation was conducted under reaction conditions reported by Zacharie et al. (Cs_2CO_3 or DBU in DMF), compounds **3** were isolated in a low 30% yield. Among the different bases used in this reaction, tetrabutylammonium fluoride (TBAF)[13] proved to be the best one for the N1-alkylation giving access to compounds **3a-d** in 60-70% yields with no trace of the N2-substitued side-products. Treating the latter intermediates with amines and AcOH/dioxane under microwave assisted conditions, it was possible to obtain the 1,4-disubsituted derivatives **4a** in acceptable yields after only 10 minutes. Alternatively, the intermediates **3a-d** were reduced with $NaBH_4$ in THF to give carbinols **5a-d** which were initially converted to the dichloro derivatives **6a-d** and then submitted to the microwave assisted C4-functionalization with different amines using the above described protocol to give the desired pyrazolo[3,4-*d*]pyrimidines **7a-d**.



Scheme 2. Alternative synthetic approach for the synthesis of substituted pyrazolo[3,4-*d*]pyrimidine analogues.

When amine hydrochlorides were used in the latter step, a different approach was required to accomplish the C4 nucleophilic substitution: the amine salt was suspended in DMF, added with an excess of triethylamine and finally treated with the intermediate **6a** under microwave irradiation at 150 °C for 10 minutes. In these conditions, both C4 nucleophilic substitution and dehydrohalogenation in the N1 linker occurred, giving the 1,4-disubstituted derivative **8a**. On the other hands, an interesting outcome in the C4 nucleophilic substitution was observed when reacting intermediate **6a** with amine **11** depending on the reaction conditions used. As previously reported, the C4 substituent of these C6-unsubstitued pyrazolo[3,4-*d*]pyrimidines seems to be solvent exposed upon binding to the target kinases and the hydrophilic amine **11** was prepared accordingly (the synthesis is reported in the SI file). Since the AcOH/dioxane substitution protocol was unsuccessful, probably due to piperazine protonation, and in order to avoid the dehydrohalogenation in N1, different reaction conditions were used in the coupling with **11**: treatment of **6b** under-microwave assisted conditions in the presence of DIPEA/*i*-PrOH gave, unexpectedly, compound **9b** as result of a dealkylative substitution instead of the expected derivative **10b**. The formation of **9b** was proved to be a direct consequence of microwave irradiation since standard heating conditions allowed to obtain compound **10b** as the only product.

In summary, a versatile approach for the synthesis of 1,4-disubstituted pyrazolo[3,4-*d*]pyrimidines has been developed. Starting from commercially available allopurinol, TBAF mediated N1-functionalization and subsequent C4 nucleophilic substitution under different reaction conditions represented the key steps for the decoration of the pyrazolo[3,4-*d*]pyrimidine scaffold. Microwave irradiation drastically reduced the reaction time need for C4 functionalization and allowed to obtain compounds (such as **9b**) inaccessible under standard heating conditions. The exploitation of this alternative synthetic protocol will allow to expand the chemical diversity around the pyrazolo[3,4-*d*]pyrimidine scaffold for the identification of novel potential anticancer agents.

Acknowledgments

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Supplementary Material

Supplementary data associated with this article can be found, in the online version, at

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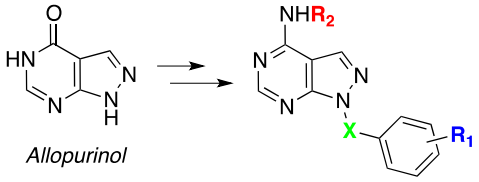

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