4-Amino-Substituted Pyrazolo[3,4-*d*]Pyrimidines: Synthesis and Biological Properties

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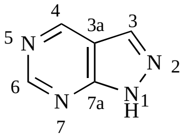
Abstract: Being the pyrazolo[3,4-*d*]pyrimidine ring system isoster with the adenine scaffold of ATP, many synthetic approaches for the construction of this bicyclic system have been developed in the last fifty years in order to identify novel bioactive molecules. The synthesis of 4-amino-pyrazolo[3,4-*d*]pyrimidine derivatives together with the functionalization of the core structure have been reviewed. The evolution of the scaffold from the pioneering work of Robins to the recent preparation of selective pharmacologically active molecules is discussed in terms of synthetic methodologies to produce biologically active compounds. With the literature on pyrazolo[3,4-*d*]pyrimidines particularly beings rich, the focus of this review deals with the construction of the ring system from pyrazoles only, reporting same selected examples in this branch of chemistry.

Key Words: Synthesis, pyrazole, pyrazolo[3,4-*d*]pyrimidine, biological activity, tyrosine kinases inhibitors.

1. INTRODUCTION

The 4-amino substituted pyrazolo[3,4-*d*]pyrimidine ring represents a very interesting scaffold for the synthesis of molecules potentially endowed with different biological activities; this structure is in fact isoster with that of the purine derivative adenine, present in ATP, that is fundamental for every aspect of cell life, and as constituent of DNA and RNA. Compounds able to modulate purine activity or metabolism and purinergic receptor activation are continuously synthesized by medicinal chemists. Chemical synthesis of these molecules can be performed starting from either the pyrimidine or the pyrazole nucleus. In this article the second type of synthetic approach will be reviewed, considering that many of the biologically active pyrazolo-pyrimidines reported in the last few years have been obtained following this route. Our attention has been focused on low molecular weight pyrazolo-pyrimidine molecules, being the synthesis of glycosylated derivatives already reported elsewhere (see for example ref. [1-3]). For reason of clarity the numbering of the pyrazolo[3,4-*d*]pyrimidine ring is reported in Fig. (**1**).



1*H*-pyrazolo[3,4-*d*]pyrimidine

Fig. (1).

Each chapter of the present review will deal with a specific class of functionalized derivatives, starting with the general procedures for the synthesis of 4-amino substituted pyrazolo[3,4-*d*]pyrimidines and then focusing on the synthesis of 3-substituted derivatives, 6-substituted derivatives and selective N1-substitution. Finally a brief overview of the high biological significance of a specific family of 4-amino-substituted pyrazolo[3,4-*d*]pyrimidines will be given. Important reviews on this topic are also referenced [1, 4].

2. PREPARATION OF PYRAZOLO[3,4-*d*]PYRIMIDINES

In the mid 50ths Ronald Robins performed the synthesis of differently substituted pyrazolo[3,4-*d*]pyrimidine systems and started the study of their biological evaluation, in particular as anti-tumor agents.

Reaction of ethoxymethylene malononitrile (**1**) and hydrazine monohydrate or differently substituted hydrazines in refluxing ethanol gave the 5-amino-1*H*-pyrazole-4-carbonitriles (**2**), which were hydrolyzed with concentrated sulphuric acid to the corresponding amides (**3**), that were finally transformed into the corresponding 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol (**4**) after treatment with boiling formamide. C4 Chlorination of the latter compounds with $POCl_3$, followed by nucleophilic displacement of the chlorine atom with primary or secondary amines in alcoholic or benzene solution at reflux afforded the amino derivatives (**6**) (Scheme **1**) [5, 6].

On the other hand, reacting the N1 unsubstituted derivative of the series (**3**) with urea or thiourea, the 1*H*-pyrazolo[3,4-*d*]pyrimidin-4,6-diol (**7**) and the corresponding 6-mercapto derivative (**8**) were respectively obtained. Treatment of the latter compound with methyl iodide led to the 6-methylthio-derivative (**9**), that submitted to the chlorination/amination protocol previously seen, gave the 4-amino substituted 6-methylthio derivatives (**11**) (Scheme **2**) [5].

Reaction of 5-amino-1*H*-pyrazole-4-carbonitrile (**2**) with formamide, urea and thiourea led to the corresponding 4-NH_2 derivatives: the adenine isoster 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**12**) (called 4-APP), the 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-ol (**13**) and the 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-thiol (**14**), respectively (Scheme **3**) [5].

Chlorination of (**7**) and of the corresponding N1 substituted derivatives, performed with $POCl_3$ and N,N-diethylaniline, afforded the 4,6-dichloro-pyrazolo[3,4-*d*]pyrimidines (**15**) in good yield (Scheme **4**). The selective replacement of the chlorine atoms with amines under carefully controlled conditions gave a variety of 4-amino and 4,6-diamino-substituted derivatives. Generally, treatment of (**15**) with aqueous or ethanolic solutions of primary or secondary amines by steam bath heating for 10 to 30 min afforded the corresponding 4-amino-6-chloro derivatives (**16**), while longer reaction times in the presence of a large excess of amines usually led to the disubstituted derivatives (**17**) [7,8].

Biological studies on Robins's pyrazolo-pyrimidines showed that the compounds possessed interesting activity including xanthine oxidase inhibition [9] and antiproliferative activity on adenocarcinoma and leukemia cell lines or in vivo tumor models [10, 11].

Following a similar synthetic approach, Schmidt and Druey reported in 1956 the synthesis of several pyrazolo[3,4-*d*]pyrimidines, starting from 5-aminopyrazole-4-carboxylates (**19a,b**), in turn obtained from ethyl(ethoxymethylene)cyanoacetate (**18**) and the appropriate hydrazine. The 4-hydroxy-derivatives (**4a,b**) were then directly obtained by reacting compound (**19a,b**) with formamide (Scheme **5**). The replacement of the C-4 cyano group with an ester

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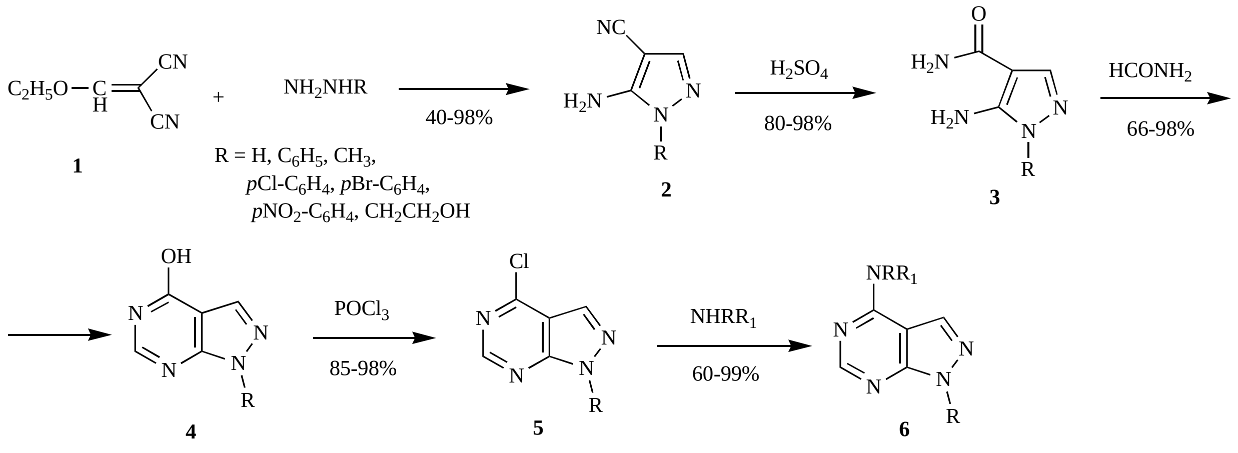
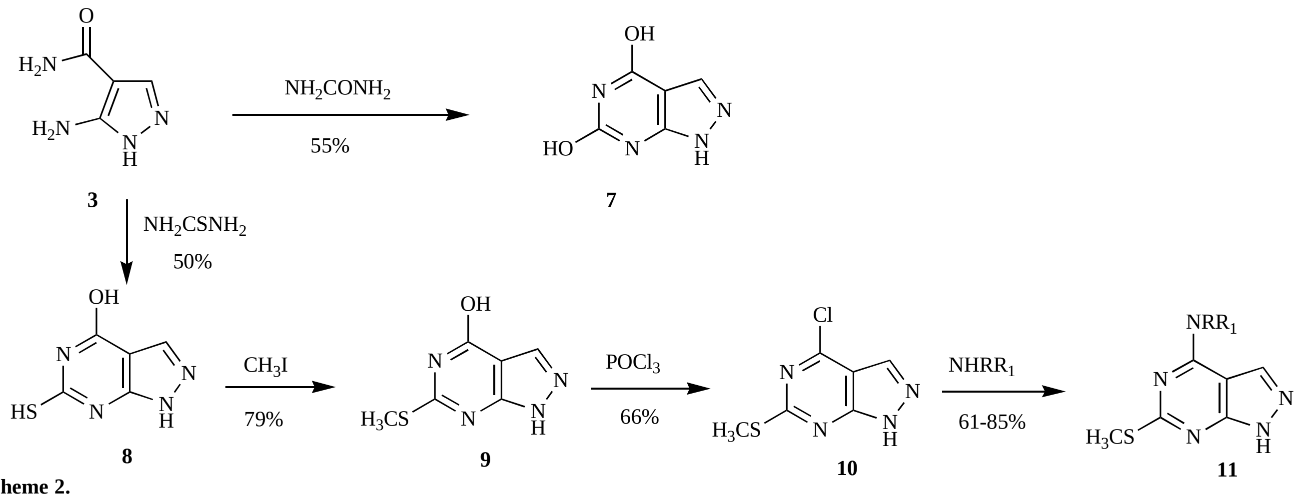
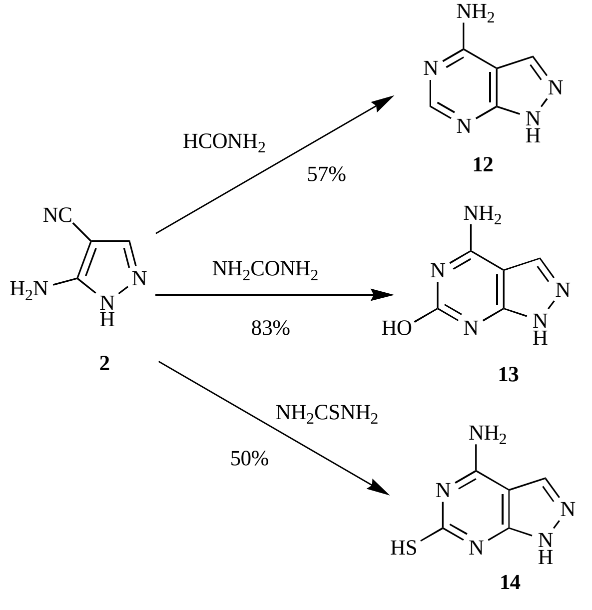


Scheme 1.



Scheme 2.



Scheme 3.

moiety within the pyrazole structure, allowed to avoid the sometimes tricky hydrolysis step [12]. This one step shortening is particularly useful when a NH_2 in C4 is not needed. Following chlorination and substitution of the chlorine atom with amines led to products analogous to (**6**).

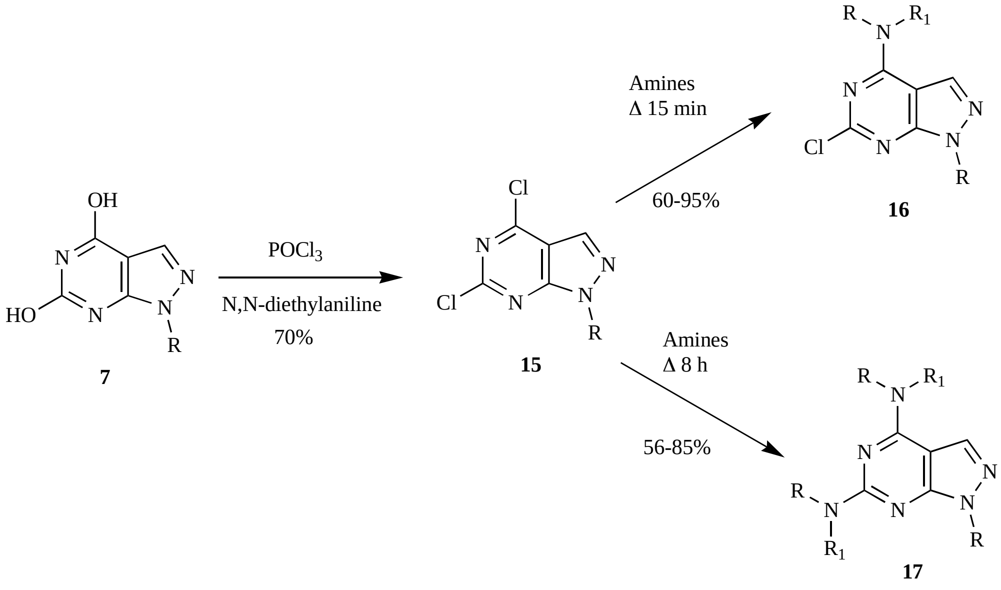
In a different synthetic approach Nagahara and Robins reported the synthesis of compounds (**4**) by reaction of the pyrazolo-amides (**3**) with ethoxymethylenemalononitrile (**1**) in refluxing DMF, thus affording the desired products in satisfactory yield (Scheme **6**) [13].

3. SYNTHESIS OF 3-SUBSTITUTED PYRAZOLO[3,4-*d*]PYRIMIDINES

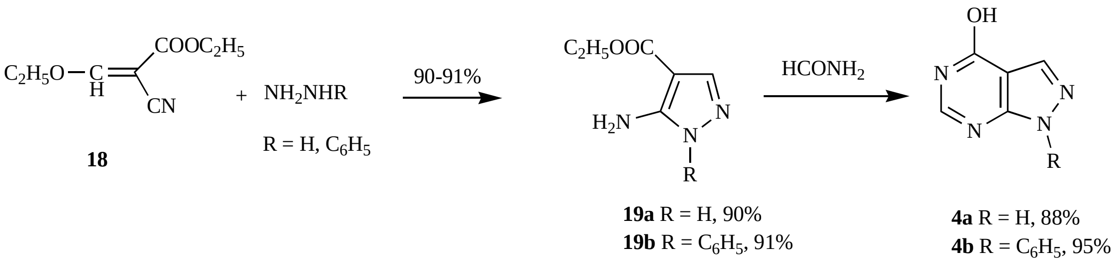
Several procedures have been reported to synthesize pyrazolo[3,4-*d*]pyrimidines bearing different substituents in position 3 of the pyrazole ring. Robins and colleagues reported the synthesis of 3-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine (**22**), by reaction of methyl-ethoxymethylenemalononitrile (**20**) and hydrazine monohydrate, followed by treatment of the corresponding cyanopyrazole (**21**) with boiling formamide in high yield [6] (Scheme **7**).

Taylor and colleagues prepared a number of new aminopyrazolo[3,4-*d*]pyrimidines, bearing a C3 cyano [14] or cyanomethyl group [15,16]. As an example, reaction of 5-amino-3-(cyanomethyl)-1-phenyl-1*H*-pyrazole-4-carbonitrile (**23**) (in turn obtained from malononitrile or malononitrile dimer and phenylhydrazine) and ethyl orthoformate led to the intermediate (**24**) which was finally cyclized with ammonia to (**25**) (Scheme **8**).

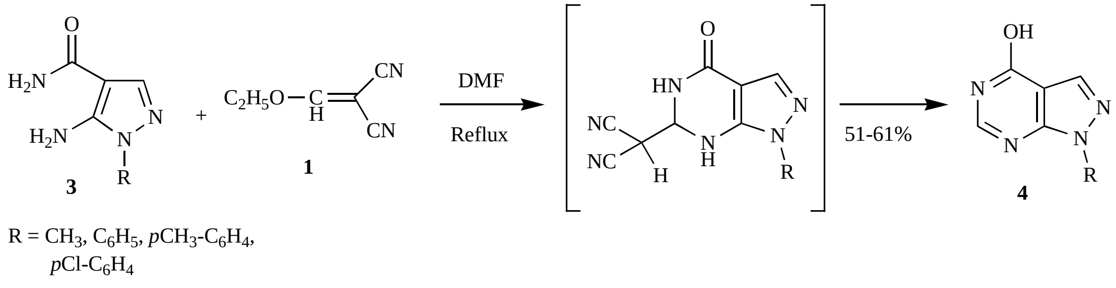
Tominaga and colleagues synthesized a variety of pyrazolo[3,4-*d*]pyrimidines starting from ketene dithioacetals, in particular from bis(methylthio)methylene malononitrile (**26a**), bis(methylthio) methylene cyanoacetamide (**26b**), anilino(methylthio)methylene



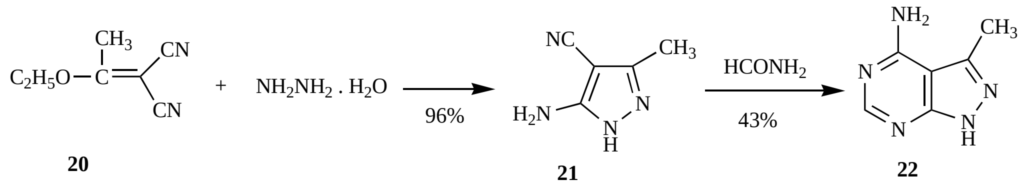
Scheme 4.



Scheme 5.



Scheme 6.

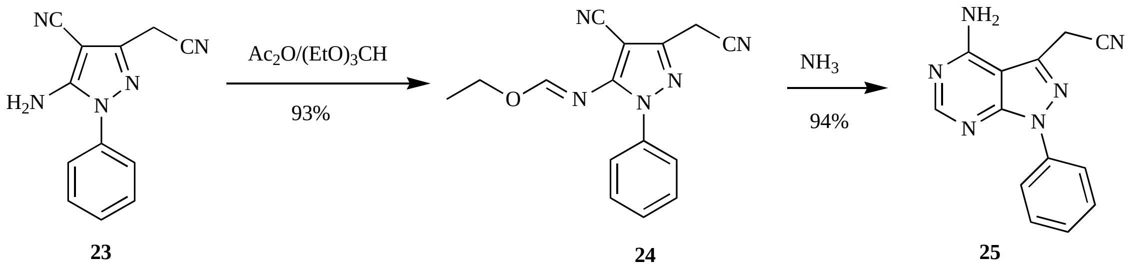


Scheme 7.

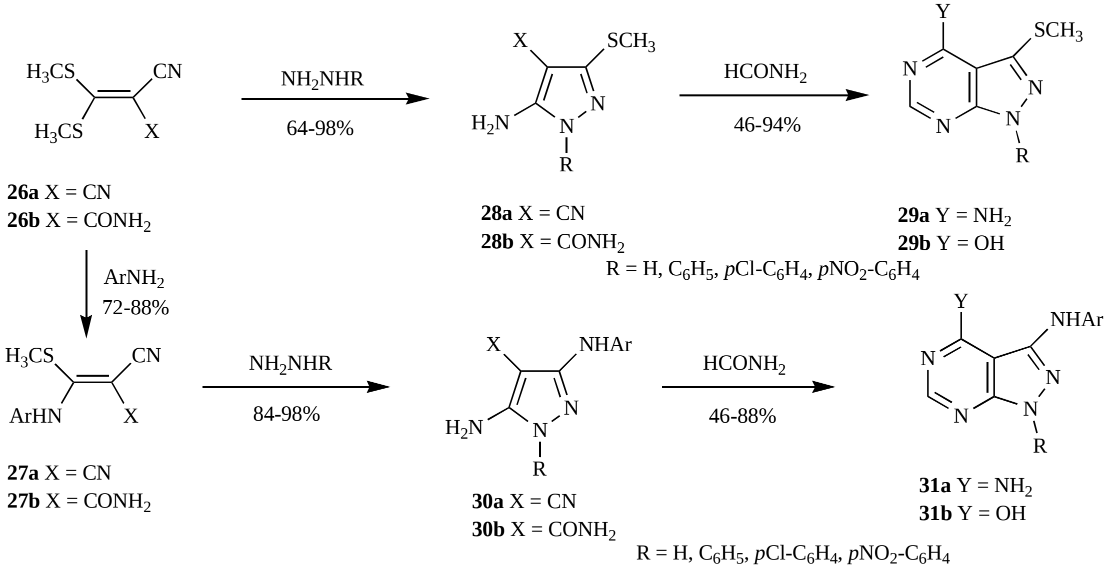
malononitriles (**27a**) and the corresponding amides (**27b**). The last two derivatives were prepared reacting (**26a**) and (**26b**) with the suitable arylamines. Reaction of (**26a,b)** and (**27a,b**) with substituted hydrazines at 100°C for 3-4 h gave the corresponding 5-amino-pyrazoles (**28a,b**) and (**30a,b**) that were in turn treated with formamide at 180°C for two hours leading to the corresponding 3-substituted pyrazolo[3,4-*d*]pyrimidine derivatives (**29a,b**) and (**31a,b**). Starting from 4-cyano-pyrazoles, the 4-amino-pyrazolopyrimidines were obtained, while the 4-hydroxy-pyrazolopyrimidines were obtained starting from pyrazole-4-carboxamide derivatives (Scheme **9**) [17].

A similar approach was used by Ram to synthesize pyrazolo[3,4-*d*]pyrimidines as potential leishmanicides, starting from derivatives (**26a**) and (**26b**) which were reacted with N1-substituted piperazines, in place of the arylamines used by Tominaga [18].

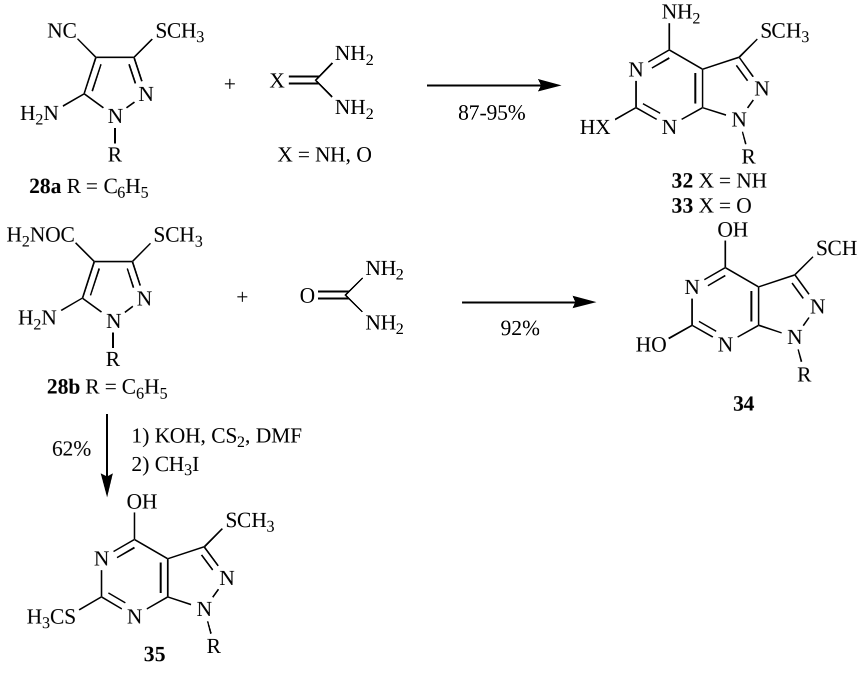
Tominaga reported also the synthesis of other substituted derivatives: reacting the N1 phenyl-substituted compound of the (**28a**)



Scheme 8.



Scheme 9.

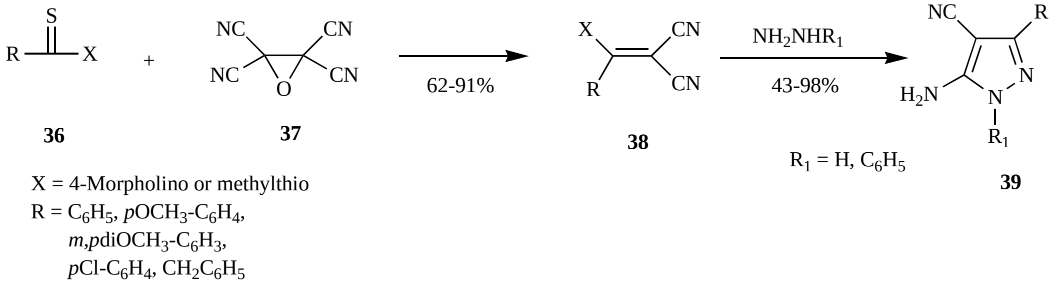


Scheme 10.

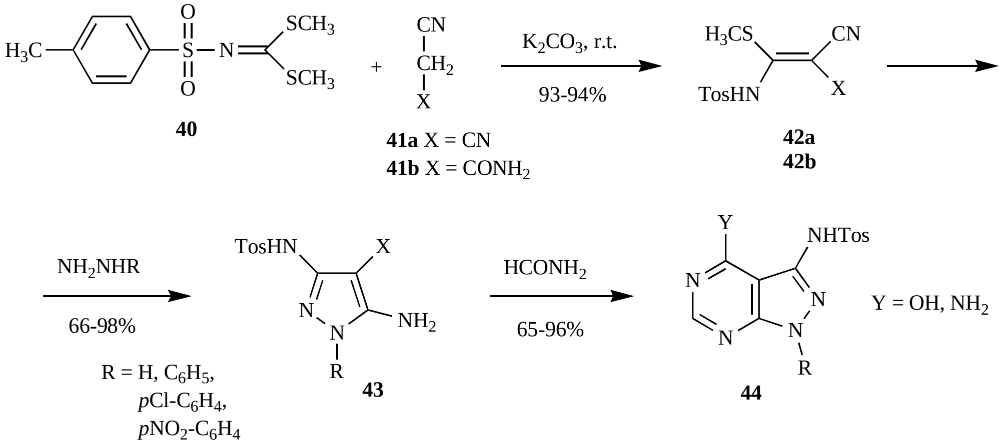
series with guanidine carbonate or with urea, the N1 phenyl pyrazolopyrimidines (**32**) and (**33**) were respectively prepared (Scheme **10**). Similarly, starting from the pyrazolo-carboxamide of the (**28b**) series, the 2,4-dihydroxy derivatives (**34**) were synthesized**,** in more than 90% yield [17].

Moreover, reacting the *o*-aminocarboxamides (**28b**) with carbon disulfide in presence of potassium hydroxide, gave the 6-methylthio analogues (**35**) in reasonable yield [17] (Scheme **10**).

Treatment of the 4-hydroxy derivatives above reported with phosphorous oxychloride gave access to the 4-chloro-pyrazolo[3,4-



Scheme 11.



Scheme 12.

d]pyrimidines which represent the key intermediates for the synthesis of the 4-amino substituted derivatives.

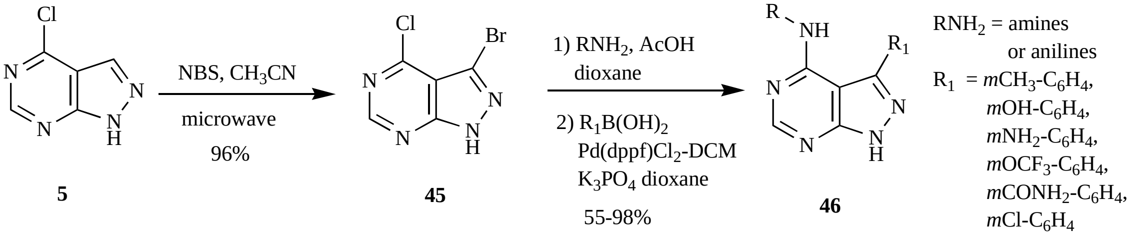
In a different article from the same Authors, the synthesis of highly functionalized dicyanoethylenes (**38**) and their application to the preparation of C3-substituted pyrazolo[3,4-*d*]pyrimidines has been reported [19]. Starting from compounds (**36**), reaction with tetracyanoethylene oxide (**37**) at room temperature in benzene gave the corresponding dicyanoethylene compounds (**38**), which were in turn reacted with hydrazine or phenyl hydrazine to obtain the 5-aminopyrazoles-4-carbonitriles (**39**) (Scheme **11**). The latter compounds were then cyclized with formamide at 180°C to give the corresponding C3-substituted 4-amino-pyrazolo[3,4-*d*]pyrimidines.

The synthesis of 3-aminopyrazolo[3,4-*d*]pyrimidines has been performed by Tominaga using as starting product the *N*-bis(methylthio)methylene-*p*-toluenesulfonamide (**40**), which reacts with malononitrile (**41a**) or cyanoacetamide (**41b**) in the presence of potassium carbonate in DMSO, affording the corresponding displacement products (**42a**) or (**42b**) in more than 90% yields. Reaction of the latter compounds with various hydrazines afforded the 5-aminopyrazole derivatives (**43**), which were in turn reacted with formamide at 200°C to give the desired pyrazolo-pyrimidines (**44**) (Scheme **12**) [20].

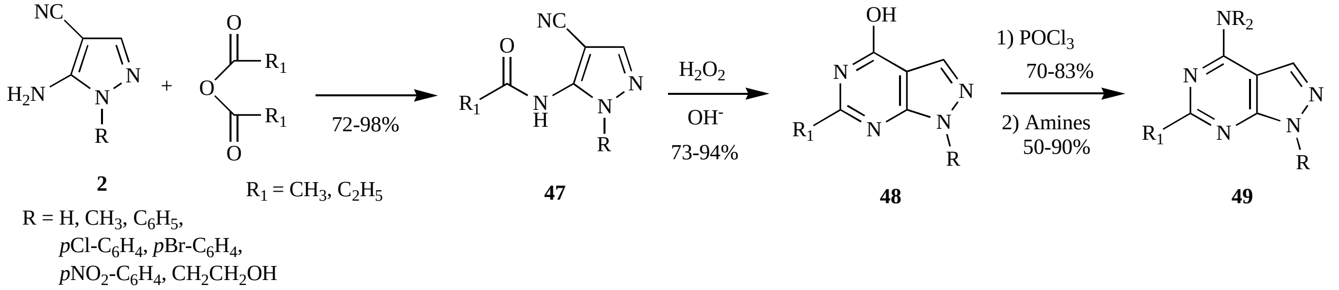
A different approach to obtain C3 substituted pyrazolo[3,4-*d*]pyrimidines has been recently reported by Schultz and Ding starting from the 3-bromo derivative (**45**), in turn obtained from the Robins's compound of the series (**5**) by treatment with N-bromosuccinimide in acetonitrile at 100°C under microwave irradiation. The application of microwave irradiation allowed to obtain high yields of the desired product (more than 95%), low side products formation and shorter reaction time. Compound (**45**) was then reacted in a one pot two step process that involves a sequential S_NAr displacement of the C4 chloro substituent with different amines in mild acidic condition (acetic acid), followed by a Suzuki coupling reaction with different boronic acids, affording the C3 substituted derivatives (**46**) (Scheme **13**). Interestingly, this approach allowed to functionalize easily the C3 position in the presence of relatively reactive functional groups (amine, amide, alcohol), that should have been protected under the strong acid or basic conditions used by the previously reported procedures [21].

4. SYNTHESIS OF 6-SUBSTITUTED PYRAZOLO[3,4-*d*]PYRIMIDINES

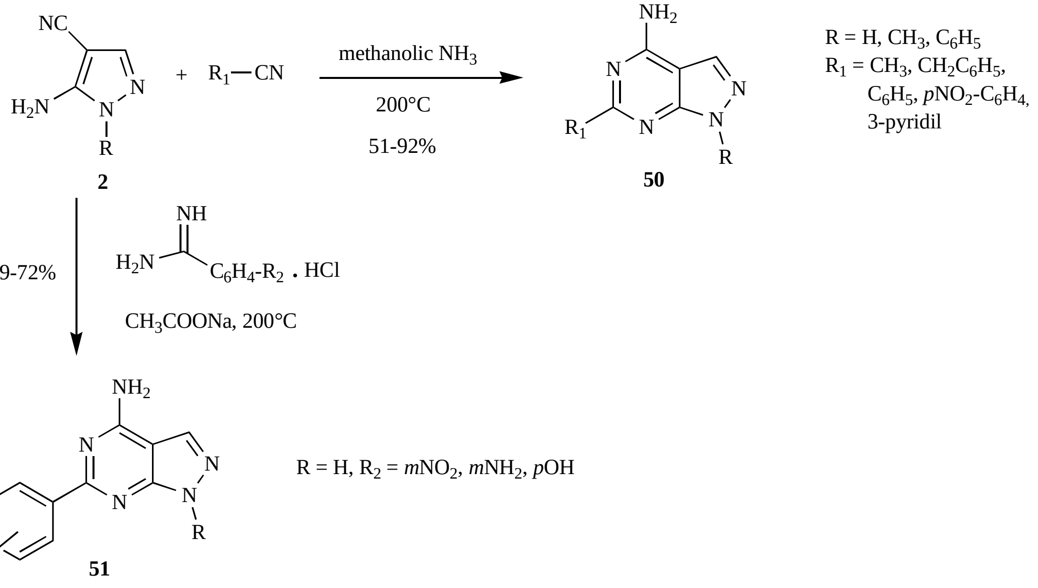
One of the first examples for the synthesis of 6-alkyl-substituted (in particular 6-methyl or 6-ethyl) pyrazolo[3,4-



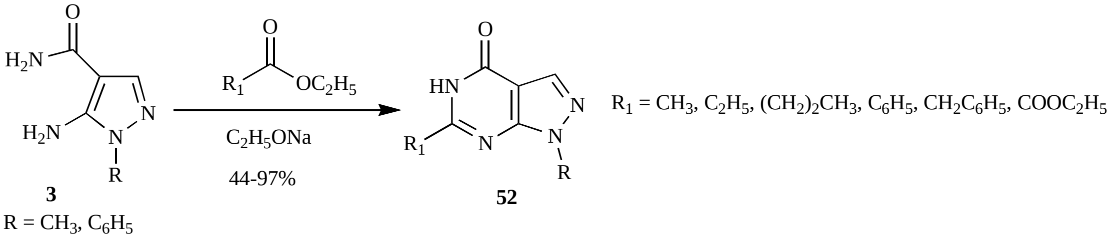
Scheme 13.



Scheme 14.



Scheme 15.



Scheme 16.

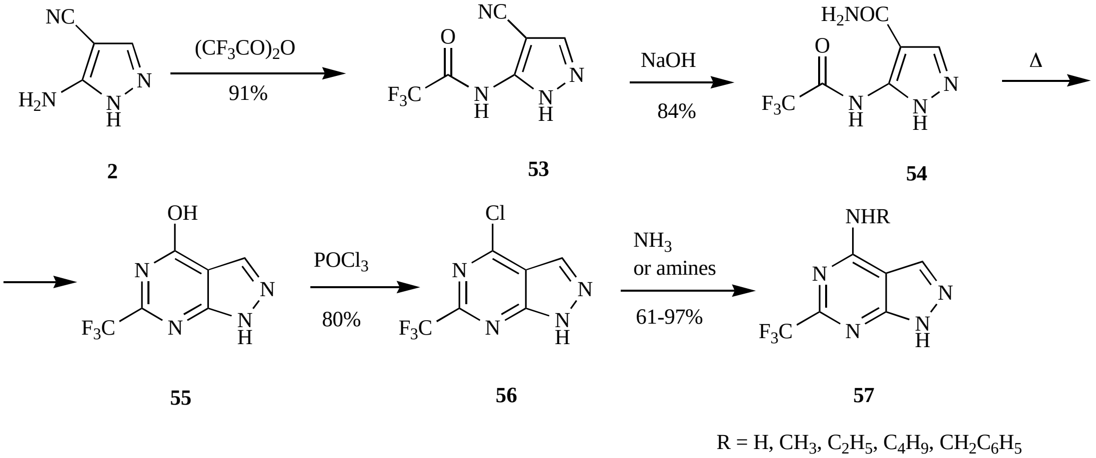
d]pyrimidines (**49**) (Scheme **14**) has been reported by Robins and colleagues [22]. The 5-amino-4-cyanopyrazoles (**2**) were acylated either by acetic or propionic anhydride to give the corresponding 5-acylamino-4-cyanopyrazoles (**47**), which were then treated with an alkaline solution of hydrogen peroxide to give the 6-alkyl-4-hydroxy-pyrazolo-pyrimidines (**48**). Finally, C-4 chlorination of (**48**) with phosphorus oxychloride and subsequent nucleophilic displacement with primary or secondary amines gave the desired compounds (**49**).

Taylor's group, during a study on the base-catalyzed condensation of aromatic and heterocyclic *ortho*-aminonitriles (**2**) with nitriles, synthesized a series of 4-amino pyrazolo[3,4-*d*]pyrimidines (**50**) bearing a substituent at C6. Starting from the 4-cyano-5-amino-pyrazoles (**2**), reaction with different nitriles in methanolic ammonia at 200°C for 20 hours, in a steel hydrogenation bomb gave the desired C6-substituted analogues (**50**) (Scheme **15**) [23].

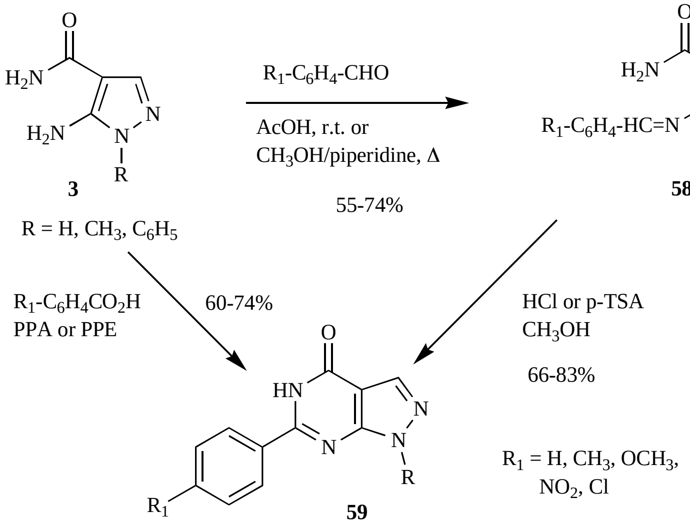
Successively Baker and Kozma synthesized 6-aryl substituted derivatives (**51**) (Scheme **15**) by reaction of (**2**) with substituted benzamidine hydrochlorides and sodium acetate at 200°C in solvent free condition [24].

An alternative methodology to obtain the 6-alkyl or 6-aryl substituted derivatives (**52**) (Scheme **16**) was represented by the easy reaction of the 1-phenyl or 1-methyl-1*H*-pyrazole-4-carboxamides (**3**) with different esters in the presence of sodium ethoxide in ethanol. The resulting derivatives (**52**) could in turn be chlorinated with $POCl_3$ and then reacted with amines, as previously reported, to give the 4-amino analogues [25].

Interestingly, 6-trifluoromethyl derivatives (**57**) were synthesized by other authors using a similar procedure. The Robins's cyanopyrazole (**2**) was reacted with trifluoro acetic anhydride, to give the intermediate (**53**), which was hydrolyzed to the corresponding amide (**54**). The latter compound was cyclized by heating at 200°C



Scheme 17.



Scheme 18.

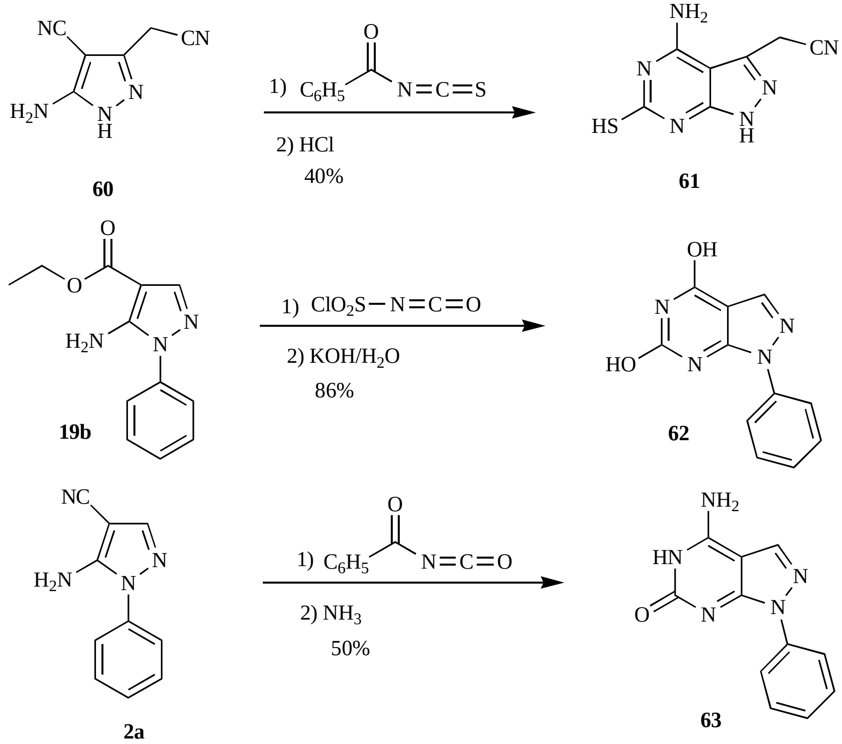
to give the pyrazolo-pyrimidine (**55**) which was first chlorinated to (**56**) and finally submitted to nucleophilic substitution with NH_3 or different amines (Scheme **17**) [26].

The amino-pyrazole carboxamides of general structure (**3**) condensed with different aromatic aldehydes afforded the 5-(*N*-arylideneamino)pyrazolo derivatives (**58**), in turn cyclized with HCl or para-toluenesulfonic acid in boiling methanol for two hours to the corresponding 6-substituted 1*H*-pyrazolo[3,4-*d*]pyrimidinones (**59**). The latter compounds can also be obtained directly by reaction of (**3**) with aromatic carboxylic acids heated in polyphosphoric acid (PPA) or polyphosphate ester (PPE) for 4-6 hours (Scheme **18**) [27]. Derivatives (**59**) represent useful intermediates for the synthesis of 4-amino substituted compounds following the already reported procedures.

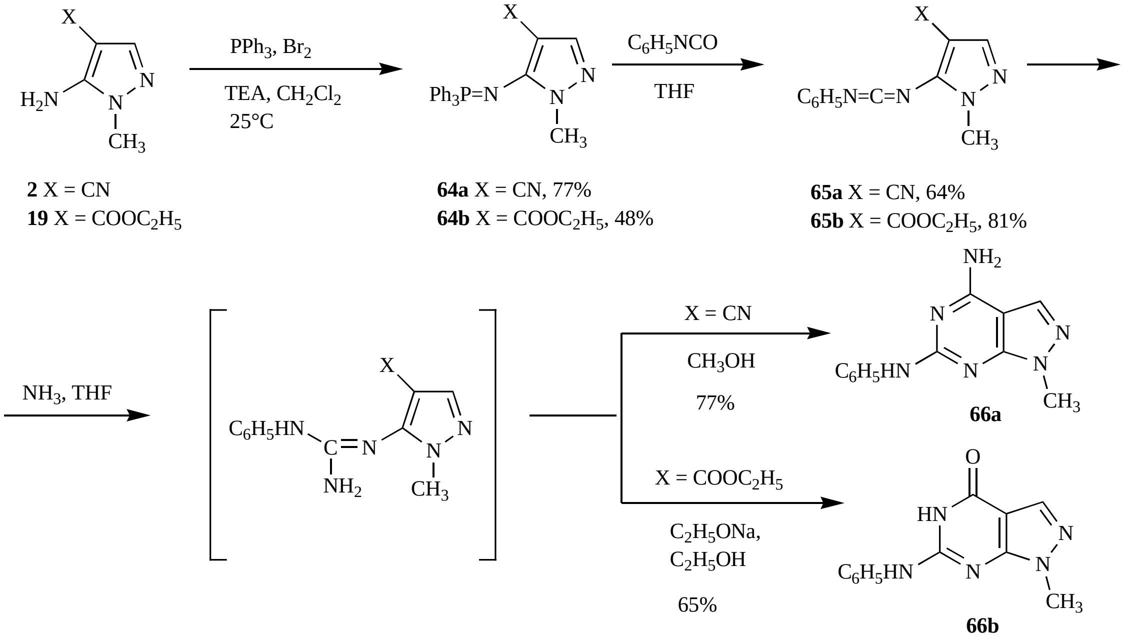
5. SYNTHESIS OF PYRAZOLO[3,4-*d*]PYRIMIDINES FROM ISOCYANATES OR ISOTHIOCYANATES

The pyrazolo[3,4-*d*]pyrimidine ring can also be synthesized by reaction of pyrazole derivatives with isocyanates or isothiocyanates, that react in a similar way as other cyclic enaminonitriles and *o*-aminonitriles [28]. The 4-amino-6-thio-pyrazolopyrimidine (**61**) has been synthesized by reacting the pyrazole (**60**) with benzoyl isothiocyanate, followed by acidification (Scheme **19**) [29]. Reaction of ethyl 5-amino-1-phenyl-1*H*-pyrazole-4-carboxylate of the series (**19**) with chlorosulphonyl isocyanate and following treatment with aqueous KOH, afforded derivative (**62**) (Scheme **19**) [30]. 4-Amino-1-phenyl-5*H*-pyrazolo[3,4-*d*]pyrimidin-6-one (**63**) has been synthesized by Quinn and colleagues in a one pot reaction involving the condensation of 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile of the series (**2**) with benzoyl isocyanate followed by annulation with ammonia, in 68% yield (Scheme **19**) [31].

Taylor and Patel applied an aza Wittig-mediated pyrimidine annulation reaction to synthesize 6-phenylamino substituted pyrazolo[3,4-*d*]pyrimidines and other fused-pyrimidine derivatives, starting from *ortho*-aminonitriles and *ortho*-aminoesters. Reaction of pyrazolo-nitrile of the series (**2**) or of the pyrazolo-ester of the series (**19**) with dibromotriphenylphosphorane, generated in situ by slow addition of bromine to a cold solution of triphenylphosphine in CH_2Cl_2 afforded the corresponding iminophosphoranes (**64a**) and (**64b**), that undergo an aza Wittig reaction with phenyl isocyanate at room temperature affording the corresponding carbodiimides (**65a**) and (**65b**) in good yields. Treatment of the latter with ammonia, followed by heating in methanol or ethanol gave, via guanidine intermediates, the final cyclized derivatives (**66a**) and (**66b**). In the case of (**66b**) the cyclization was greatly facilitated by addition of sodium ethoxide (Scheme **20**) [32].



Scheme 19.



2 X = CN
19 X = $COOC_2H_5$

64a X = CN, 77%
64b X = $COOC_2H_5$, 48%

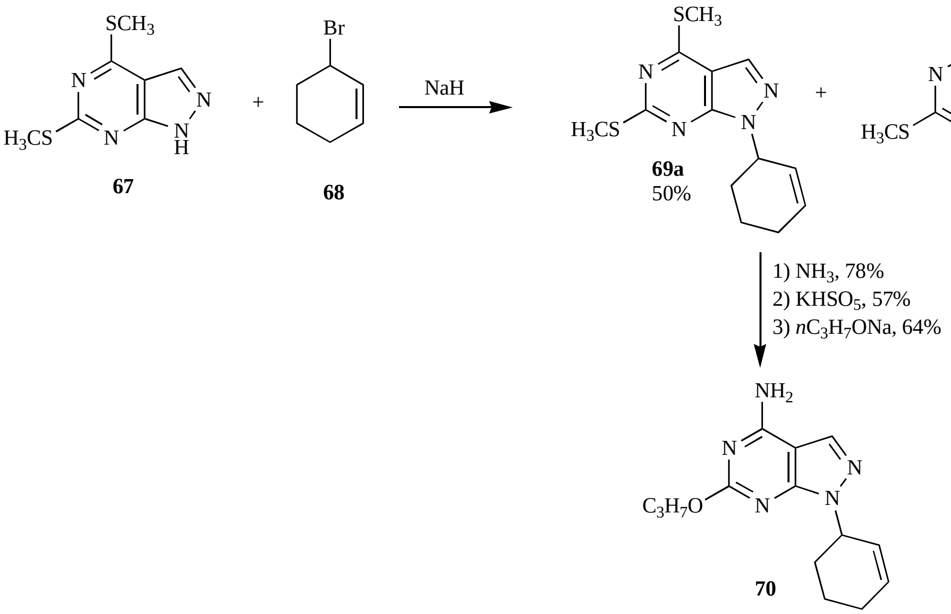
65a X = CN, 64%
65b X = $COOC_2H_5$, 81%

Scheme 20.

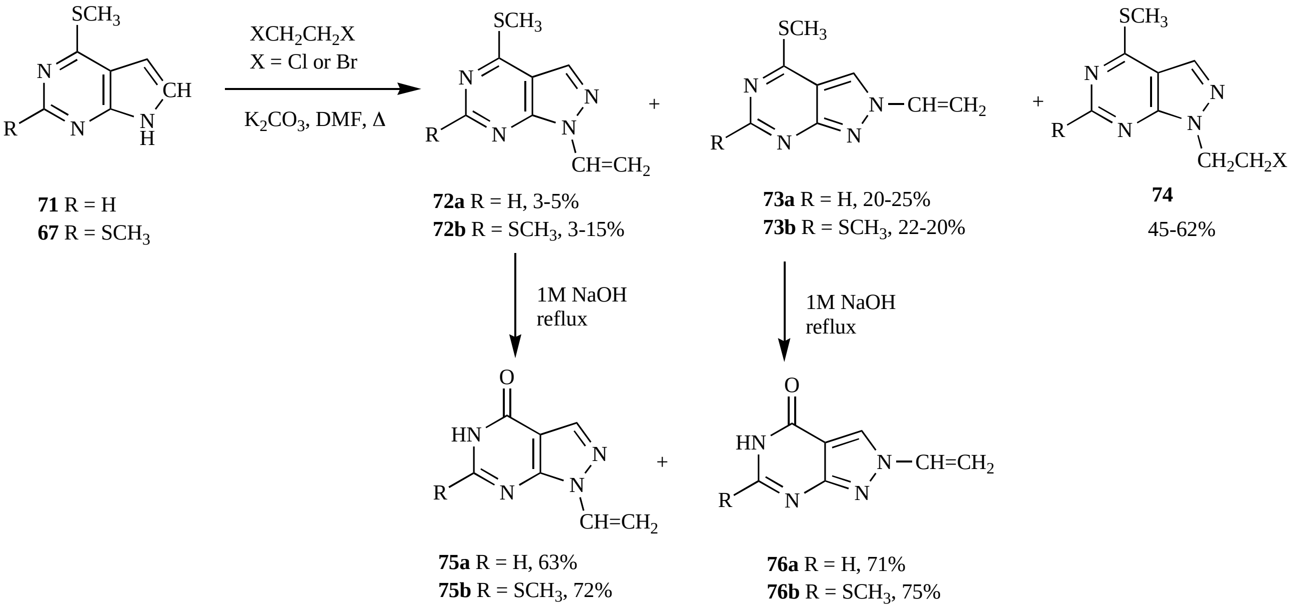
6. SELECTIVE N1-SUBSTITUTION OF PYRAZOLO[3,4-*d*]PYRIMIDINES

Procedures leading to N1 substituted derivatives usually start from the corresponding substituted hydrazines, but also the direct N1 substitution of the pyrazolo[3,4-*d*]pyrimidine ring is sometimes possible, especially for short chain alkylation. Usually, a concurrent N2 substitution occurs but, in particular reaction conditions, the N1 substitution product predominates and can be isolated via standard chromatographic procedures. Being this topic a very wide field of research, only a few significant examples will be herein reported.

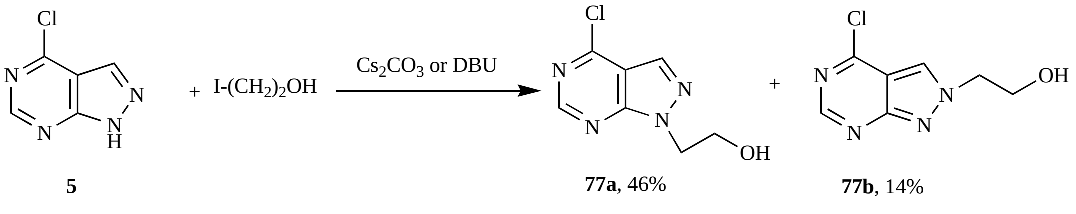
Bhakuni and colleagues synthesized a number of pyrazolo[3,4-*d*]pyrimidines starting from the Taylor's 4,6-bis(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**67**) [33] by treatment with sodium hydride and 3-bromo-1-cyclohexene (**68**). The reaction led to the N1 and N2 substituted products (**69a**) and (**69b**) in 50 and 10 % yield respectively. Compound (**69a**), purified on a silica gel column, was successively treated with ammonia, $KHSO_5$ and sodium propoxide to give the desired compound (**70**) (Scheme **21**) [34]. Differently, reacting (**67**) with methyl, ethyl, propyl or benzyl iodide in the presence of sodium hydride, the N2 substituted derivatives were obtained as major products [35].



Scheme 21.



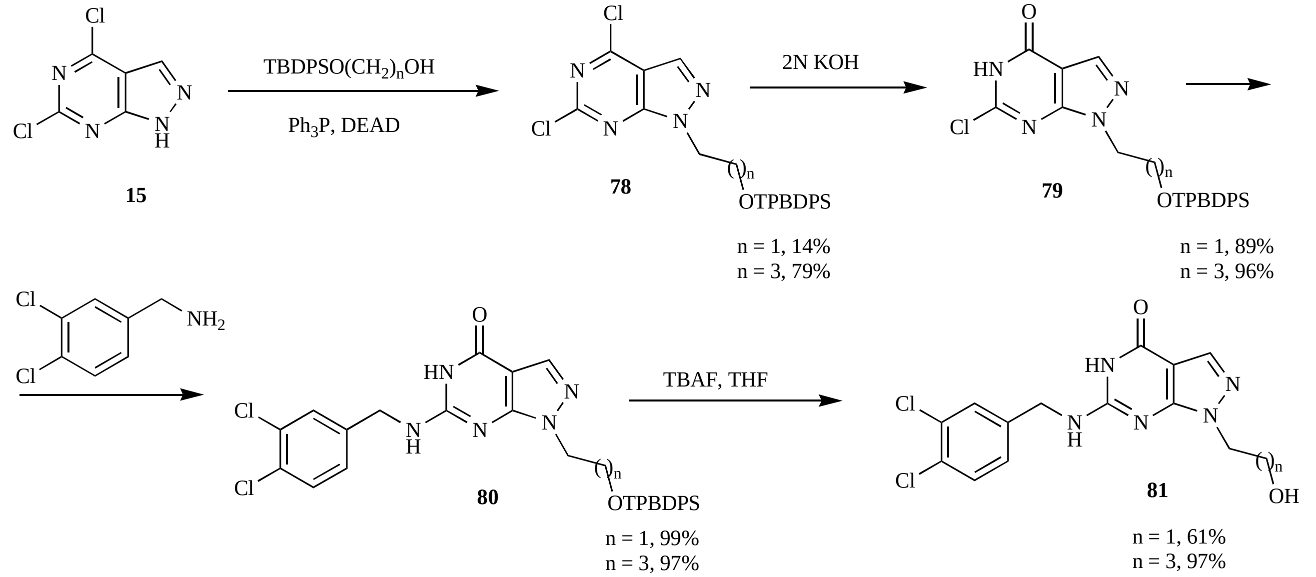
Scheme 22.



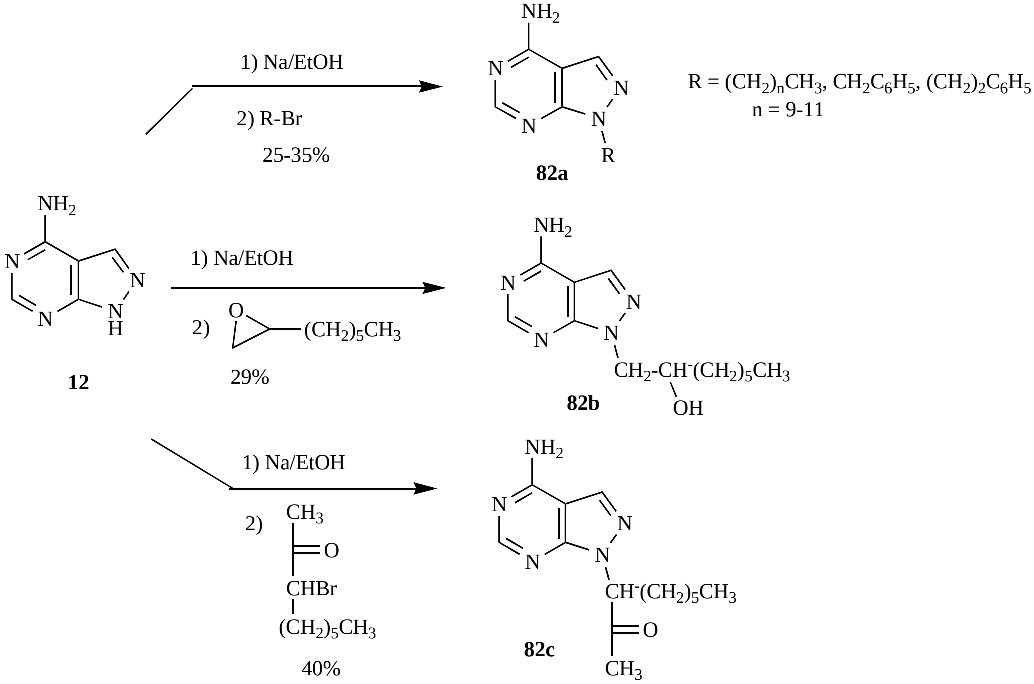
Scheme 23.

The treatment of derivatives (**67**) or (**71**) with 1,2-dibromo or 1,2-dichloroethane, in presence of potassium carbonate in DMF, gave an easily separable mixture of the N1 vinyl derivatives (**72a,b**), the N2 vinyl derivatives (**73a,b**) and the side reaction products (**74**). Reaction of the separated isomer (**72**) and (**73**) with 1M NaOH at reflux afforded the corresponding pyrazolopyrimidinones (**75a,b**) and (**76a,b**) (Scheme **22**) [36].

Zacharie and coworkers reported the alkylation of the derivative of the series (**5**) with 2-iodoethanol in the presence of cesium carbonate or DBU in dry DMF at 0°C leading to the N1 isomer (**77a**) in about 46% yield and N2 isomer (**77b**) in 14-20% yield (Scheme **23**). The same reaction, performed with the sodium salt of (**5**) (prepared with sodium hydride) and using the same alkylating agent in acetonitrile gave only traces of the N1 isomer [37].



Scheme 24.



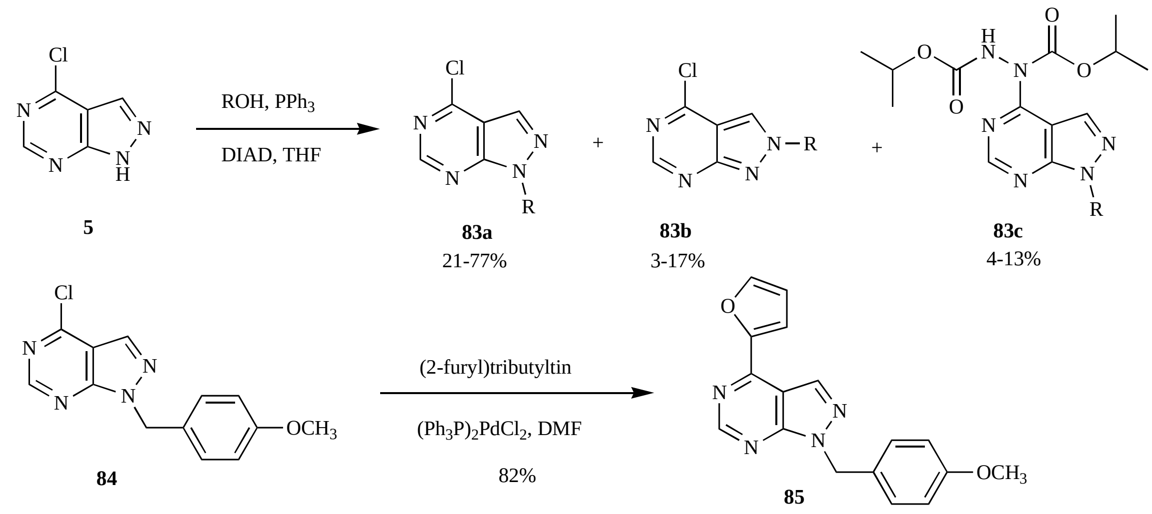
Scheme 25.

An interesting example of N1 substituted pyrazolo-pyrimidines was reported by Merck researchers, in the synthesis of the N1-hydroxyalkyl-substituted derivatives (**81**), endowed with inhibitory activity on *Staphylococcus Aureus* DNA polymerase III. The 4,6-dichloropyrazolo[3,4-*d*]pyrimidine of the series (**15**) underwent Mitsunobu reaction with protected hydroxyalkyl derivatives, Ph_3P, DEAD in THF at room temperature to give intermediates (**78**). Selective hydrolysis with 2N KOH afforded the 6-chloropyrazolo[3,4-*d*]pyrimidin-4-ones (**79**), which was in turn reacted with 3,4-dichloro benzylamine yielding the derivatives (**80**), finally deprotected to (**81**) (Scheme **24**) [38].

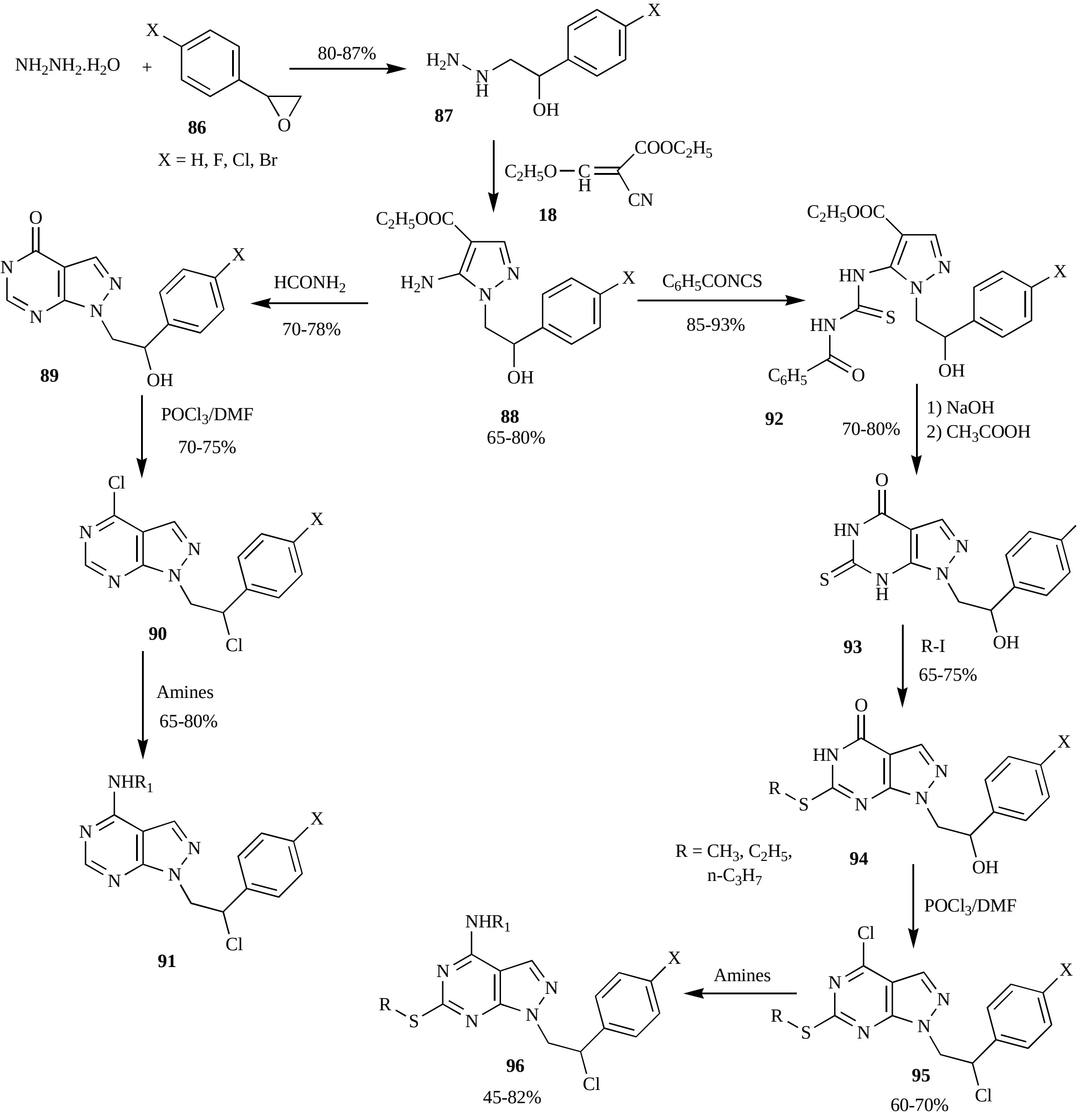
Da Settimo and colleagues have recently synthesized a series of N1 differently substituted 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidines (**82a**-**c**) as adenosine deaminase inhibitors, by reaction of 4-APP (**12**) sodium salt (prepared with sodium in ethanol) with alkyl halides or oxiranes in DMF. The products were purified by flash chromatography and obtained in yields ranging from 25 to 40% (Scheme **25**) [39].

Efficient N1-selective alkylation of 4-chloropyrazolo[3,4-*d*]pyrimidine (**5**) has been recently reported by Gundersen [40]. Reaction of unsubstituted derivative of the series (**5**) with a number of alcohols under Mitsunobu conditions led to the predominant formation of the N1 substituted derivatives (**83a**), in yields generally ranging from 21 to 77%. In some cases N2 substituted derivatives (**83b**) together with the C4 substituted product (**83c**) have been isolated in small percentage (Scheme **26**).

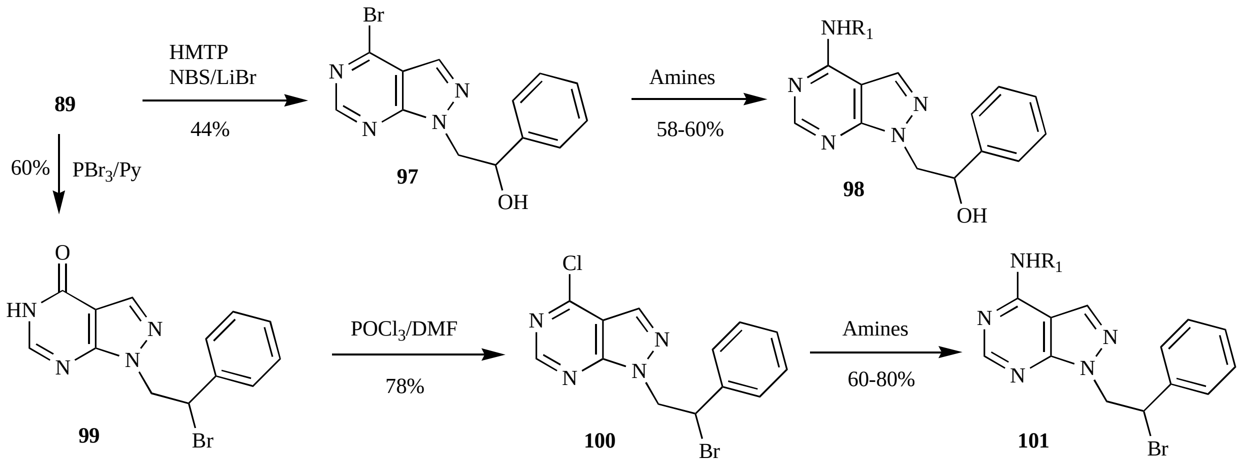
The same Authors also reported the C4 functionalization of the compound (**84**) (prepared by Mitsunobu reaction with (**5**) and *p*-methoxy-benzyl alcohol) by reaction with (2-furyl)tributyltin, $(Ph_3P)_2PdCl_2$ in DMF, to obtain the derivative (**85**) in 82% yield,



Scheme 26.



Scheme 27.



Scheme 28.

representing the first example of a palladium-catalyzed coupling reaction on a 4-halopyrazolo[3,4-*d*]pyrimidine (Scheme **26**) [40].

7. BIOLOGICAL ACTIVITY OF 4-AMINO-SUBSTITUTED PYRAZOLO[3,4-*d*]PYRIMIDINES

N1-substituted or N1-unsubstituted 4-amino-pyrazolo[3,4-*d*]pyrimidines showed a broad spectrum of biological activities including adenosine receptor antagonism [41-47], anticonvulsant activity [48], tyrosine kinases inhibition [49], cyclin-dependent kinase 2 (CDK2) inhibition [50] cyclooxygenase-2 inhibition together with anti-angiogenic activity [51], anti-enterovirus [52], and antimicrobial activity [53].

Our attention will be focused on a library of 4-amino substituted pyrazolo[3,4-*d*]pyrimidines acting as dual inhibitors of Src/Abl, two cytoplasmatic tyrosine kinases involved in the development of chronic myeloid leukemia [54].

In the continuing efforts to find new anticancer agents, we have recently synthesized a family of highly functionalized pyrazolo[3,4-*d*]pyrimidines (**91**, **96**, **98**, **101**). These compounds have been synthesized starting from the phenyloxiranes (**86**) that reacting with hydrazine monohydrate, led to the corresponding 2-hydrazinoethanols (**87**). Reaction of the latter with ethyl(ethoxymethylene) cyanoacetate (**18**) afforded the ethyl esters of 5-amino-1*H*-pyrazole-4-carboxylic acids (**88**). Reaction of these derivatives with an excess of formamide at 190°C gave the pyrazolo-pyrimidinones (**89**) which were transformed into the dichloro derivatives (**90**) by treatment with the Vilsmeier complex ($POCl_3$:DMF, 1:1). Regioselective substitution of the chlorine at C4 with an excess of various amines, afforded the products (**91**) in good yields **(**Scheme **27**). Alternatively, refluxing compounds (**88**) with benzoyl isothiocyanate for 12 h in THF gave the intermediates (**92**) which were cyclized to the pyrazolo[3,4-*d*]pyrimidinones (**93**) by treatment with 2M NaOH, followed by acidification with acetic acid. Alkylation of the thio group at C6 with the appropriate alkyl iodide in refluxing THF afforded the 6-alkylthio derivatives (**94**), which were in turn treated with the Vilsmeier complex to obtain the dihalogenated compounds (**95**). Reacting the latter derivatives with an excess of various amines afforded the products (**96**) in good yields (Scheme **27**) [55, 56].

Compound (**97**) was prepared following the Beal and Véliz procedure [57] by treatment of (**89**) with a mixture of hexamethylphosphorous triamide/N-bromosuccinimide (HMPT/NBS) in acetonitrile at -20°C followed by addition of LiBr and refluxing for 10 hours. It is interesting to point out that the secondary OH on the N1 side chain remains unaltered with this procedure. The C4 bromoderivative (**97**) was treated with the appropriate amines to give the final compounds (**98**). Reaction of the pyrimidinone (**89**) in DMF with a solution of phosphorous tribromide, pyridine and toluene at room temperature for 3 days led to the intermediate (**99**), bearing a bromine atom on the N1 side chain. The latter was in turn chlorinated at C4 by treatment with the Vilsmeier complex, to afford the intermediate (**100**) which was finally reacted with an excess of various amines to obtain the derivatives (**101**) in high yields (Scheme **28**) [58,59].

Compounds (**91**) and (**96**), bearing a 2-chloro-2-phenylethyl N1 side chain showed a submicromolar to nanomolar activity toward isolated Src as well as antiproliferative activity toward the epidermoid (A431) and breast cancer (BC-8701) cell-lines (that overexpress Src) blocking Src phosphorylation and inducing apoptosis.

The most potent compounds of the series reduced proliferation, migratory ability and adhesive capacity of PC3 carcinoma cells [60] and inhibit the growth of human SaOS-2 xenograft tumor model in nude mice [61]. Moreover nanomolar concentrations of these c-Src inhibitors have a highly selective antiangiogenic effect [62].

Based on the fact that compounds acting as c-Src inhibitors often showed also activity toward Bcr-Abl, a set of these pyrazolo[3,4-*d*]pyrimidine derivatives has been tested on Abl isolated enzyme and on a panel of leukemia cell lines. The studied compounds were able to inhibit Bcr-Abl and c-Src phosphorylation, induced apoptosis and, as the activation of Src and Abl is an important step in the progression of leukemia cells (in particular, chronic myeloid leukemia), reduced cell proliferation [63].

CONCLUSIONS

The most common methods for the synthesis of pyrazolo[3,4-*d*]pyrimidine scaffold have been reported starting from the earlier work of Robins, when the biological behaviour of these compounds was still in an embryonic state, up to recent days. We have described how the evolution of chemical methodologies, which led to many new derivatives, tightly accompanied the evolution of modern biology, pharmacology and modern medicine. It is fascinating how the functionalization of the pyrazolo[3,4-*d*]pyrimidine scaffold can give rise to selective inhibitors for many different biological targets, which number is in rapid evolution.

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