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Original article

A novel and efficient synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives and the study of their anti-bacterial activity

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ABSTRACT

In this work 4-amino-6-aryl-2-phenyl pyrimidine-5-carbonitrile derivatives were synthesized through a one-pot, three-component reaction of an aldehyde, malononitrile and benzamidine hydrochloride, in the presence of magnetic nano Fe₃O₄ particles as a catalyst under solvent-free conditions. 3-Amino-6-aryl-2-phenylpyrazolo[3,4-*d*]pyrimidine derivatives were prepared through an efficient and environmentally friendly reaction between 4-amino-6-aryl-2-phenylpyrimidine-5-carbonitrile derivatives and hydrazine hydrate and their antibacterial activity has been evaluated.

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1. Introduction

Fused pyrimidine derivatives have shown diverse biological activities. Among them, pyrazolo[3,4-d]pyrimidines as purine analogs have attracted considerable interest due to their remarkable pharmacological properties. These compounds were designed and synthesized as potent and selective kinase inhibitors [1,2], antileishmanial, antitrypanosomal [3], antibacterial [4] and antiviral agents [5] and adenosine A_{2A} receptor antagonists [6]. Some literature examples exist for the synthesis of pyrazolo[3,4d]pyrimidines [7–13]. These methods suffer from some drawbacks such as lengthy reaction sequences, expensive reagents, and/or tedious workup procedures. These interesting bioactivity of pyrazolo[3,4-d]pyrimidines motivated us to search for a more facile synthetic route for the 3-amino-6-aryl-2-phenylpyrazolo[3,4-d]pyrimidine derivatives. Herein, we wish to report a straightforward synthesis of novel pyrazolo[3,4-d]pyrimidines derivatives using 6-amino-4-aryl-2-phenylpyrimidine-5-carbonitrile derivatives as the starting materials. This synthetic protocol

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has some advantages, including benign reaction conditions, fewer synthetic steps to reach pyrazolo[3,4-*d*]pyrimidine derivatives compared to other reports from readily available materials, simple work-up, high overall yields of the products and the simultaneous conversion of nitro to amino groups, which provides an opportunity to synthesize more complex structures with additional reaction steps.

2. Experimental

Melting points were recorded on a Buchi B-540 apparatus. IR spectra were recorded on an ABB Bomem Model FTLA200-100 instrument. ¹H NMR and ¹³C NMR spectra were measured on a Bruker DRX-300 spectrometer at 300 MHz and 75 MHz using TMS as an internal standard. Chemical shifts (δ) were reported relative to TMS, and coupling constants (J) were reported in hertz (Hz). Mass spectra were recorded on a Shimadzu QP 1100 EX mass spectrometer with 70 eV ionization potential. Elemental analyses of new compounds were performed with a Vario EL III 0 Serial No. 11024054 instrument and their results favorably agreed with the calculated values. The bacterial strains were isolated from different organs of patients at the Microbiological Laboratory of Ghaem Hospital of Medical University of Mashhad, Iran.

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Scheme 1. Reaction used for the synthesis of pyrimidine-5-carbonitrile derivatives.

2.1. General procedure for the synthesis of 4a-4m

Malonitrile (1 mmol), aldehyde (1 mmol), benzamidine hydrochloride (1 mmol), magnetic nano Fe_3O_4 (0.06 g), were ground and placed in a test tube. The reaction mixture was stirred under heating for 1–1.5 h at 100 °C and monitored by TLC, (EtOAc/ petroleum ether, 1:2). After the completion of the reaction, the reaction mixture was cooled to ambient temperature, EtOAc was added, and the nano Fe_3O_4 was separated using an external magnet, then the mixture was concentrated in vacuo to obtain a solid product. The residues were further purified by crystallization from EtOH. It is worth to mention that the nano Fe_3O_4 could be reused three times without any significant loss of activity.

2.2. General procedure for the synthesis of 5a-5i

To a solution of 4-amino-6-aryl-2-phenyl pyrimidine-5-carbonitrile derivatives (1 mmol) in EtOH (5 mL) was added hydrazine hydrate (0.5 mL) and the mixture was heated under reflux. After the completion of the reaction (monitored by TLC, EtOAc: petroleum ether, 4:1) the reaction mixture was cooled to ambient temperature and water (10 mL) was added. The solid materials were filtered off and filtrate was concentrated and recrystallized from ethanol.

Selected analytical data: 3-Amino-6-(4-chlorophenyl)-2-phenylpyrazolo[3,4-d]pyrimidine (5b): Pale yellow powder; mp: 267 °C; IR (KBr, cm⁻¹): ν 3449, 3325, 3307; ¹H NMR (300 MHz, DMSO- d_6): δ 12.82 (s, 1H, NH), 8.48–8.49 (m, 2H), 8.04–8.07 (d, 2H, J = 8.3 Hz), 7.66–7.69 (d, 2H, J = 8.3 Hz), 7.51–7.53 (m, 3H), 5.19 (s, 2H, NH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 159.9, 159.8, 156.1, 147.8, 137.6, 136.0, 135.3, 131.2, 130.6, 128.8, 128.6, 128.1, 100.7; MS (m/z): 321 (54); Anal. Calcd. for C₁₇H₁₂ClN₅: C, 63.46; H, 3.76; Cl, 11.02; N, 21.77; Found: C, 63.58; H, 3.75; N, 21.65. 3-Amino-6-(3-aminophenyl)-2-phenylpyrazolo[3,4-d]pyrimidine (5g): Pale yellow powder; mp: 311.5 °C; IR (KBr, cm⁻¹): ν 3430, 3416, 3341; ¹H NMR (300 MHz, DMSO-*d*₆): δ 12.00 (s, NH, br), 8.47–8.50 (m, 2H), 7.50– 7.52 (m, 3H), 7.23-7.28 (m, 1H), 7.13 (s, 1H), 7.00-7.03 (d, 1H, J = 7.5 Hz), 6.77–6.80 (d, 1H, J = 7.4 Hz), 5.42 (s, 2H, NH₂), 5.09 (s, 2H, NH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 162.1, 160.0, 155.8, 149.2, 147.6, 138.0, 137.7, 130.4, 129.3, 128.5, 128.0, 116.1, 115.7, 113.7,100.4; MS (m/z): 302 (100), 301 (64); Anal. Calcd. for C₁₇H₁₄N₆: C, 67.54; H, 4.67; N, 27.80; Found: C, 67.47; H, 4.56; N, 28.18.

2.3. Determination of minimum inhibitory concentration (MICs)

The MICs were assayed by the tube dilution test method, introduced by National Committee for Clinical Laboratory Standards (NCCLS). A serial of dilutions of tested compounds (final concentration of 300–0.8 μ mol/L) were added to the tested bacteria in Mueller–Hinton broth (0.5 mL) in 24-well plates and were incubated at 37 °C for 24 h (5 × 10⁵ CFU/mL). After sufficient amount of incubation (24 h), the wells were examined for turbidity as an indicator for growth of the bacteria. For further confirmation, an aqueous solution of 2,3,5-triphenyltetrazolium chloride (0.5%; 100 μ L) was added to the wells. The lowest concentration of the

compound that prevented the bacteria growth (<99%) was designated as the minimum inhibitory concentration (MIC). Growth was present in the medium control but was absent from the inoculum control.

3. Results and discussion

Initially, aromatic aldehyde **1**, malononitirile **2**, and benzamidine hydrochloride **3**, were mixed and heated in the presence of magnetic Fe₃O₄ nanoparticles as a catalyst under solvent-free conditions at 100 °C (Scheme 1, Table 1). The products **4a**–**m** were all prepared with excellent yields at 100 °C in 1–1.5 h. Both aromatic aldehydes with electron donating substituents (Table 1, entries 7 and 13) and electron-withdrawing substituents (Table 1, entries 6, 9 and 10) showed significant reactivity in this process.

Then, 6-amino-4-(4-chlorophenyl)-2-phenylpyrimidine-5-carbonitrile (1 mmol) (Table 1, entry 2, **4b**), hydrazine hydrate (1 mL) and EtOH (5 mL) were mixed and refluxed for 8 h. The reaction was completed and only one new spot appeared on the TLC plate. After aqueous work-up, a pure pale yellow solid was obtained from the reaction mixture whose full characterization by spectroscopic methods (IR, ¹H NMR, ¹³C NMR, and mass spectra) revealed that pyrazolo[3,4-*d*]pyrimidine **5b** was obtained. In order to determine the amount of hydrazine needed in this process, the reaction of pyrimidine **4b** was performed using 0.5 mL of hydrazine hydrate and the same product was again obtained.

However, using 0.065 mL (1 mmol) of hydrazine hydrate did not result in the formation of product **5b** and only the starting material was recovered. So, the amount of 0.5 mL was chosen as the optimal amount of hydrazine hydrate for each mmol of pyrimidine derivatives. In the next step a model reaction (Scheme 1, Ar = 4-Cl-Ph) was performed in different solvents under different conditions and the results showed that using EtOH as solvent under reflux is the best condition. In order to examine the scope and generality of this protocol for the synthesis of other fused pyrazolo[3,4-*d*]pyrimidine derivatives, the pyrimidine derivatives shown in Table 1 were also subjected to this process

Table 1

Synthesis of pyrimidines $\bf 4a-m$ under solvent free conditions catalyzed by magnetic $\rm Fe_3O_4$ nanocatalyst.

Entry	Product	Ar	Yield ^a (%)	Mp (°C)	Lit. mp (°C)
1	4a	Ph	98	214	210-212 [15]
2	4b	4-Cl-Ph	96	222	222 [15]
3	4c	4-Br-Ph	94	234-236	236[14]
4	4d	2,3-diCl-Ph	96	231-234	-
5	4e	2-Cl-Ph	96	200-202	196 [16]
6	4f	4-CN-Ph	98	299-300	-
7	4g	4-Me-Ph	96	211	210 [15]
8	4h	2,4-diCl-Ph	96	174	170–17 [18]
9	4 i	3-NO ₂ -Ph	96	201-202	-
10	4j	4-NO ₂ -Ph	96	215-217	215 [16,17]
11	4k	3-indolyl	90	230-232	-
12	41	4-CH₃CONH-Ph	95	245	243-244 [17]
13	4m	4-MeO-Ph	95	213	213 [15]

^a Isolated yield.

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Tab	le	2	
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Synthesis of 5a-i in EtOH under reflux condition.

Entry	Pyrimidine	Product	Time (h)	Yield (%) ^a	Mp (°C)
1	$ \begin{array}{c} & \underset{N \\ Ph}{ } \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	$ \begin{array}{c} & & \\ & & $	8	97	261–264
2	$\begin{array}{c} \text{CI} & \text{CN} \\ & \text{H} & \text{NH}_2 \\ & \text{N} & \text{N} \\ & \text{Ph} \end{array}$	$\begin{array}{c} CI & \longrightarrow \\ H_2N & NH \\ H_2N & NH \\ H_1N & NH \\ N & Ph \\ Dh \\ \mathbf{5b} \end{array}$	8	95	267
3	$\overset{\text{Br}}{\underset{\substack{\parallel\\ \parallel\\ N\\ Ph}}{\overset{\text{CN}}{\underset{Ph}{\overset{\text{NH}_2}{}}}}$	$\begin{array}{c} Br \\ H_2N \\ H_2N \\ N \\ N \\ Ph \\ 5c \end{array}$	8	91	271
4	$ \begin{array}{c} & \underset{Cl}{\overset{CN}{\underset{H}{\underset{H}{\underset{H}{\underset{H}{\underset{H}{\underset{H}{\underset{H}{$	$ \begin{array}{c} & \overset{H_2N}{\underset{Cl}{}{\underset{N}{}{\underset{Ph}{}{}{\underset{Ph}{}{}{}{\underset{Sd}{}{}}}} \\ & \overset{N}{\underset{Ph}{}{\underset{Sd}{}{}{\underset{Sd}{}{}{\underset{NH}{}{\underset{Ph}{}{}{\underset{Sd}{}{}{\underset{Sd}{}{}{\underset{NH}{}{\underset{Sd}{}{}{\underset{Sd}{}{}{\underset{Sd}{}{}{\underset{Sd}{\underset{Sd}{}{\underset{Sd}{\underset{Sd}{}{\underset{Sd}{\underset{Sd}{}{\underset{Sd}{\underset{Sd}{}{\underset{Sd}{\underset{Sd}{}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{}{\underset{Sd}{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{Sd}{\underset{Sd}{\underset{Sd}{\underset{Sd}{Sd}{\underset{Sd}{Sd}{\underset{Sd}{\underset{Sd}{Sd}{\underset{Sd}{Sd}{\underset{Sd}{Sd}{\underset{Sd}{Sd}{Sd}{Sd}{\underset{Sd}{Sd}{Sd}{Sd}{Sd}{\underset{Sd}{Sd}{Sd}{Sd}{Sd}{Sd}{\underset{Sd}{Sd}{Sd}{Sd}{Sd}{Sd}{Sd}{Sd}{Sd}{Sd}$	1	98	212
5	$\overset{\mathrm{NC}}{\substack{\parallel\\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ $	$H_2N = N$ NH $N = N$ Ph 5e	3	85	261
6	$\begin{array}{c} & \\ & \\ & \\ & \\ & \\ & \\ & \\ & \\ & \\ & $	$\begin{array}{c} & & \\$	2	97	215
7	O_2N NH_2	$H_{2N} \xrightarrow{H_{2}N} N_{NH} \xrightarrow{N}_{Ph} 5g$	5	90	311.5
8	O_2N CN NH_2 $N \rightarrow NH_2$ Ph	H_2N H_2N NH H_2N $H_$	5	88	223–229
9	CN NH2 N Ph	O H ₂ N NH NH NH Ph 5i	8	95	211

^a Isolated yield.

(Scheme 2, Table 2). The results of the synthesis of the fused pyrimidine derivatives are presented in Table 2. Some unanticipated observations are made from the data in Table 2.

When the pyrimidine derivatives with a 4-(3-nitrophenyl) or a 4-(4-nitrophenyl) group (Table 1, entries 9 and 10) were used, the reaction led to the formation of pyrazolopyrimidine derivatives with the nitro group on the phenyl ring having been reduced to an amino group (Table 2, entries 7 and 8). Such a transformation has been reported before in the literature for the reduction of nitro to amino group using hydrazine as reductant [19]. Similarly, when a

pyrimidine derivative with a 4-(4-cyanophenyl) substituent (Table 1, entry 6) was used, we again obtained a pyrazolopyrimidine derivative lacking a cyano group on the phenyl ring as can be seen in product **5a** (Table 2, entry 5). When bulky aryl substituents were placed at position 4 of the pyrimidine ring (Table 1, entries 4, 8, 11 and 12), the addition of hydrazine hydrate led to decomposition of the pyrimidine to the benzylidiene malononitrile and benzamidine intermediates rather than the formation of related pyrazolo[3,4-*d*]pyrimidine derivatives.

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Scheme 2. Synthesis of 3-amino-6-aryl-2-phenylpyrazolo[3,4-d]pyrimidine derivatives under optimized reaction conditions.

Table 3

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Antibacterial activity of some novel pyrazolopyrimidine derivatives.

Compounds		
	Enterococcus raffinosus	Staphylococcus aureus
5a	259.1	136.2
5c	12.3	3.8
5b	>300	190.3
5i	14.2	4.2
5f	>300	202.5
5h	82.8	34.5
5g	82.8	34.5
5d	56.1	20.2
Penicillin G	93.5	24.4

The inhibitory property of the synthetic compounds against two Gram-negative strains of bacteria, *Pseudomanas aeruginosa* and *Klebsiella pneumoniae*, and two Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus raffinosus* were evaluated. The results are presented in Table 3 and penicillin G was used as a reference antibacterial agent. Compounds **5a–i** exhibited weak to moderate antibacterial activity only against the Gram-positive pathogens (MIC = 3.8–300 μ mol/L). The bactericidal activity of the tested compounds against *E. raffinosus* (MIC = 3.8–202.5 μ mol/L) was higher than that determined against the *S. aureus* (MIC = 12.3– 300 μ mol/L). Amongst those compounds tested, **5c** and **5i** were the most active agents. The MIC values of 3.8 μ mol/L and 4.2 μ mol/L against *E. raffinosus* and 12.3 μ mol/L and 14.2 μ mol/L against *S. aureus* were obtained. Compounds **5a**, **5b** and **5f** were not inactive against the mentioned bacteria (MIC > 150 μ mol/L).

4. Conclusion

In summary, we have developed a novel, efficient protocol for the synthesis of fused pyrazolo[3,4-*d*]pyrimidine derivatives from the corresponding pyrimidine-5-carbonitrile derivatives. The spontaneous conversion of a nitro to an amino group that provides an opportunity to synthesize more complex structures with additional reaction steps is a unique advantage of this protocol. Also these compounds were evaluated for biological activity and **5c** and **5i** showed interesting antibacterial activity compared to the reference penicillin G.

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