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Microwave-assisted synthesis and antibacterial evaluation of new derivatives of 1,2-dihydro-3*H*-pyrazolo[3,4-*d*]pyrimidin-3-one

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Abstract: Synthesis of new 1,2-dihydro-3*H*-pyrazolo[3,4-*d*] pyrimidin-3-ones **4–6** starting with ethyl 4-hydroxy-2-methylthio-pyrimidine-5-carboxylate (**1**) under classical heating and microwave-induced conditions is reported. The antibacterial activities of the synthesized compounds were evaluated using chloramphenicol and streptomycin as reference drugs.

Keywords: antibacterial; microwave; pyrazolo[3,4-*d*] pyrimidin-3-one; synthesis.

Dedication: In the memory of Professor Mohammad Rahimizadeh

Introduction

Pyrazolo[3,4-*d*]pyrimidines and related fused derivatives are a pharmaceutically important class of compounds [1]. They exhibit diverse pharmaceutical activities such as antifungal [2], antibacterial [2, 3], neuroleptic [4], antihypertensive [5] and antileishmanial [6] properties. Also, pyrazolopyrimidines have been exploited as ATP competitive inhibitors of kinases [7], cAMP phosphodiesterase [8] and DNA polymerase [9]. Some of these compounds have demonstrated antitumor activities [10] by inhibiting different types of enzymes such as cyclin-dependent kinase [10, 11, 12] adenosine deaminase [12] glycogen synthase kinase-3 [13] and epidermal growth factor receptor protein tyrosine kinase [14].

Most synthetic approaches to pyrazolopyrimidines start from substituted pyrimidines or pyrazoles [15]. The cyclization of pyrimidines with hydrazines provides pyrazolopyrimidines via the construction of the fused pyrazole ring [16]. Alternatively, pyrazolopyrimidines can also be synthesized through the construction of the pyrimidine ring using substituted pyrazoles [10]. Recently, some new methods have been reported based on using microwave irradiation [16], ionic liquids [17], supported solid catalysts [17, 18] and under solvent-free conditions [19].

As part of our ongoing program aimed at developing new protocols for preparation of biologically active heterocyclic compounds [20], we wish to report the result of our investigations on the synthesis and antimicrobial activities of new derivatives of pyrazolo[3,4-*d*] pyrimidin-3(2*H*)-ones.

Results and discussion

The starting material, ethyl 4-hydroxy-2-methylthio-pyrimidine-5-carboxylate (**1** in Scheme 1), was prepared according to the previously published method [21]. The treatment of **1** with morpholine, pyrolidine and piperidine, led to the selective S_NAr displacement of the thiomethyl moiety and gave the respective pyrimidines **2a–c**. Then, the chloropyrimidines **3a–c** were obtained by heating compounds **2a–c** in a mixture of PCl_5 and $POCl_3$ (1:14) [22–24]. Compounds **3a–c** served as direct precursors to the desired products **4a–c, 5a–c** and **6a–c** by the reaction with hydrazine, methylhydrazine and phenylhydrazine, respectively. Classical synthesis of pyrazolo[1,5-*a*]pyrimidines **4–6** was performed by heating the reactants in ethanol in the presence of sodium ethoxide (Method A in Scheme 1). The observed different regioselectivities for the reaction

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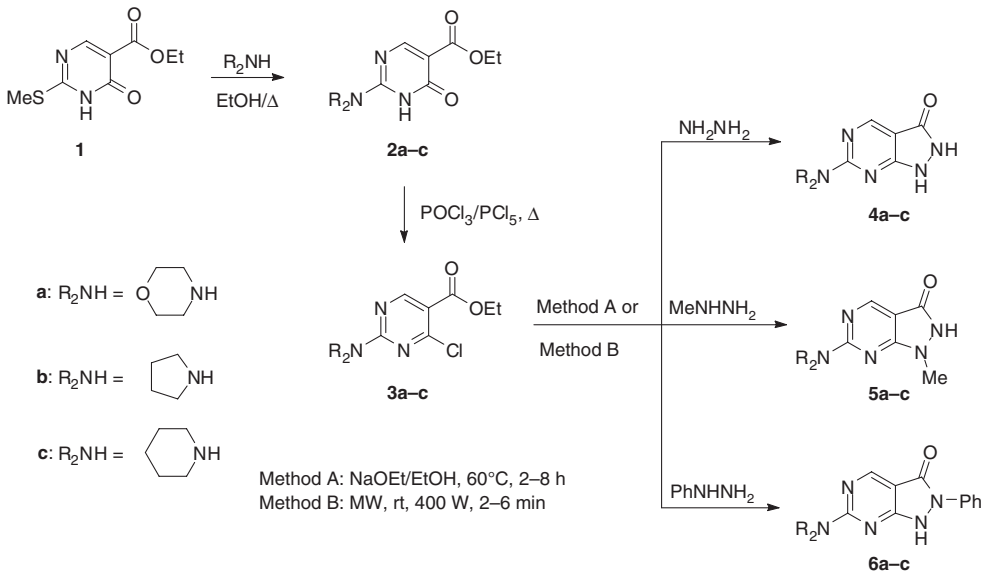

Scheme 1

with methylhydrazine to give product **5** and for the reaction with phenylhydrazine leading to product **6** are in full agreement with the literature data [25–27]. A similar, microwave-assisted organic synthesis (MAOS) [28, 29] is designated in Scheme 1 as Method B. Comparison of the results of the two methodologies reveals that the use of microwave irradiation greatly reduces the reaction time (from 2–8 h to 2–6 min) and significantly increases the yield. The structures of products **4–6** are fully supported by the results of IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis. A literature survey [30, 31] reveals that synthetic approaches to pyrazolopyrimidines mostly involve a heterocyclization reaction of pyrimidine moiety onto the pyrazole core [15]. Our synthetic protocol is different and based on the construction of the pyrazole moiety at the pyrimidine core.

Compounds **4–6** were tested for antibacterial activity against Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778) and Gram-negative (*Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 9027) bacteria. The results of antibacterial properties of active compounds are shown in Table 1. The results indicate that compound **6c** shows the strongest antibacterial properties against all tested bacteria. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of compound **6c** against *E. coli* are significantly better than those of chloramphenicol, the reference drug. Inhibition activity of **6c** against *P. aeruginosa* is the same as that of streptomycin. Finally, the antibacterial activity of **6c** against *S. aureus* is higher than that of chloramphenicol and is equal to streptomycin.

Conclusion

In summary, we applied classical conditions and MAOS to the preparation of pyrazolo[3,4-*d*]pyrimidin-3(2*H*)-ones

Table 1 Antibacterial activities (MIC and MBC μg/mL) of active compounds.

Entry	*B. subtilis* ATCC 12711		*S. aureus* ATCC 25923		*E. coli* ATCC 25922		*P. Aeruginosa* ATCC 9027	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5a	416.6	844	78	104	104	208	416	416
5b	500	500	250	500	250	250	500	500
6a	500	500	62.5	125	250	500	500	500
6b	125	250	62.5	125	125	125	250	250
6c	62.5	125	31	62.5	31	31	125	125
Chloramphenicol	250	250	125	250	125	250	250	250
Streptomycin	62.5	62.5	31	31	250	250	125	125

4a–c, 5a–c and **6a–c**. In all cases, reaction times and yields of products were dramatically improved under the MAOS protocol. Compound **6c** has a very good antimicrobial activity compared to chloramphenicol and streptomycin.

Experimental

Melting points were recorded on an Electrothermal type 9100 melting point apparatus. The IR spectra were obtained in KBr discs on an Avatar 370 FT-IR Thermo Nicolet instrument. The ^{1}H NMR (400 MHz) and the ^{13}C NMR (100 MHz) spectra were recorded in DMSO-d_6 on a Bruker Avance DRX-400 spectrometer. The electron impact mass spectra were scanned on a Varian Mat CH-7 instrument at 70 eV. Elemental analyses were performed on a Thermo Finnigan Flash EA microanalyzer. The *in vitro* antibacterial activities of the compounds were determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) recommended MIC protocol with minor modifications [32]. The minimum bactericidal concentrations (MBC) we also determined [33]. Volumes of 5 µL of every well without growth were transferred to agar plates and incubated at 37°C for 24 h. The lowest concentration of the synthetic compounds where no viable bacteria were identified was taken as MBC [33].

General procedure for the synthesis of compounds 3a-c

Phosphorus pentachloride (2.7 mmol, 0.5 g) was added to a suspension of ethyl 2-(substituted)amino-6-oxo-1,6-dihydropyrimidine-5-carboxylate (3.5 mmol, 1.0 g) in phosphorus oxychloride (7 mL). After the mixture had been heated under reflux for 3 h, the residue was cooled and poured onto ice (~30 g). The resulting solid was filtrated and crystallized from petroleum ether.

Ethyl 4-chloro-2-(morpholin-1-yl)dihydropyrimidine-5-carboxylate (3a) Yield 68%; white solid; mp 82°C; ^{1}H NMR: δ 1.39 (t, 3H, *J* = 7.2 Hz, CH_3), δ 3.77 (t, 4H, *J* = 5.1 Hz, 2-CH_2N), 3.93 (t, 4H, *J* = 5.1 Hz, 2-CH_2O), 4.36 (q, 2H, *J* = 7.1 Hz, CH_2), 8.84 (s, 1H, CH-pyrimidine); IR: ν 2974, 2913, 2859, 1724, 1696, 1587 cm^{-1}; MS: *m/z* 271 (M^+). Anal. Calcd for $C_{11}H_{14}ClN_3O_3$: C, 48.63; H, 5.19; N, 15.47. Found: C, 48.55; H, 5.24; N, 15.62.

Ethyl 4-chloro-2-(pyrrolidin-1-yl)-pyrimidine-5-carboxylate (3b) Yield 67%; white solid; mp 60°C; ^{1}H NMR: δ 1.39 (t, 3H, *J* = 7.2 Hz, CH_3), 2.04 (m, 4H, 2-CH_2), 3.67 (t, 4H, *J* = 6.3 Hz, 2-CH_2N), 4.36 (q, 2H, *J* = 7.2 Hz, CH_2), 8.85 (s, 1H, CH-pyrimidine); IR: ν 2978, 2855, 1716, 1586 cm^{-1}; MS: *m/z* 255 (M^+). Anal. Calcd for $C_{11}H_{14}ClN_3O_2$: C, 51.67; H, 5.52; N, 16.43. Found: C, 51.85; H, 5.37; N, 16.65.

Ethyl 4-chloro-2-(piperidin-1-yl)-pyrimidine-5-carboxylate (3c) Yield 70%; white solid; mp 50°C; ^{1}H NMR: δ 1.36 (t, 3H, *J* = 7.2 Hz, CH_3), 1.65 (m, 6H, 3-CH_2), 3.85 (t, 4H, *J* = 5.1 Hz, 2-CH_2N), 4.32 (q, 2H, *J* = 7.2 Hz, CH_2), 8.79 (s, 1H, CH-pyrimidine); IR: ν 3113, 3039, 2960, 2921, 2859, 1746, 1641, 1585 cm^{-1}; MS: *m/z* 269 (M^+). Anal. Calcd for $C_{12}H_{16}ClN_3O_2$: C, 53.43; H, 5.98; N, 15.58. Found: C, 53.55; H, 5.94; N, 15.72.

General procedures for the synthesis of 4a-c, 5a-c and 6a–c

Method A A solution of sodium ethoxide obtained from sodium metal (1 mmol, 0.023 g)] and absolute ethanol (5 mL) was treated with **3a–c** (1 mmol) and hydrazine, methylhydrazine or phenylhydrazine (1.2 mmol). The mixture was heated on a water bath at 60°C and, after completion of the reaction as was monitored by TLC using $CHCl_3$/MeOH (20:1), the solvent was removed under reduced pressure. The solid residue was dissolved in water and the solution acidified with acetic acid. The resulting precipitate was filtered, washed with water (2 × 30 mL), dried and crystallized from methanol to give the product **4a–c, 5a–c** or **6a–c**.

Method B A mixture of compound **3a–c** (1 mmol) and an excess amount of the appropriate hydrazine (0.1 mL) was subjected to microwave irradiation in the absence of solvent (maximum power 400W during 2–6 min at room temperature) using a focused microwave reactor (Milestone). After the completion of the reaction, water (5 mL) was added. The solid product was collected by filtration and crystallized from methanol to give the corresponding compounds **4a–c, 5a–c** or **6a–c**.

6-(Morpholin-1-yl)-1,2-dihydro-3*H*-pyrazolo[3,4-*d*]pyrimidin-3-one (4a) Reaction time 5 h (method A), 4 min (method B); yield 68% (A), 84% (B); yellow solid; mp 297–299°C; ^{1}H NMR: δ 3.64 (t, 4H, *J* = 4.8 Hz, 2-CH_2N), 3.74 (t, 4H, *J* = 4.4 Hz, 2-CH_2O), 8.66 (s, 1H, CH-pyrimidine), 11.19 (br s, 2H, 2-NH, D_2O exchangeable); ^{13}C NMR: δ 44.7, 66.4, 98.7, 153.9, 157.6, 158.8, 161.5; IR: ν 3120, 2965, 2917, 2847, 1631, 1564 cm^{-1}; MS: *m/z* 221 (M^+). Anal. Calcd for $C_9H_{11}N_5O_2$: C, 48.86; H, 5.01; N, 31.66. Found: C, 48.91; H, 4.98; N, 31.72.

6-(Pyrrolidin-1-yl)-1,2-dihydro-3*H*-pyrazolo[3,4-*d*]pyrimidin-3-one (4b) Reaction time 6 h (A), 5 min (B); yield 58% (A), 80% (B); yellow powder; mp 325–328°C (decomp.); ^{1}HNMR: δ 1.96 (m, 4H, 2-CH_2), 3.53 (t, 4H, *J* = 6.8 Hz, 2-CH_2N), 8.77 (s, 1H, CH-pyrimidine), 12.64 (br s, 2H, 2-NH); ^{13}C NMR: δ 25.3, 47.2, 112.5, 159.9, 160.0, 160.9, 166.3; IR: ν 3158, 2974, 2884, 1635, 1556 cm^{-1}; MS: *m/z* 205 (M^+). Anal. Calcd for $C_9H_{11}N_5O$: C, 52.67; H, 5.40; N, 34.13. Found: C, 52.71; H, 5.37; N, 34.09.

6-(Piperidin-1-yl)-1,2-dihydro-3*H*-pyrazolo[3,4-*d*]pyrimidin-3-one (4c) Reaction time 6 h (A), 4 min (B); yield 75% (A), 87% (B); brown powder; mp 298–300°C; ^{1}H NMR: δ 1.50 (m, 4H, 2-CH_2),1.62 (m, 2H, CH_2) 3.78 (t, 4H, *J* = 5.2 Hz, 2-CH_2N), 8.61 (s, 1H, CH-pyrimidine), 10.94 (br s, 1H, NH, D_2O exchangeable), 11.21 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 24.7, 25.7, 45.1, 98.1, 130.6, 154.1, 160.0, 161.4; IR: ν 3154, 3131, 3043, 2931, 2853, 1630, 1564 cm^{-1}; MS: *m/z* 219 (M^+). Anal. Calcd for $C_{10}H_{13}N_5O$: C, 54.78; H, 5.98; N, 31.94. Found: C, 54.73; H, 6.03; N, 32.01.

1-Methyl-6-morpholino-1,2-dihydro-3*H*-pyrazolo[3,4-*d*]pyrimidin-3-one (5a) Reaction time 2 h (A), 2 min (B); yield 73% (A), 89% (B); white powder; mp 231–235°C; ^{1}H NMR: δ 3.53 (s, 3H, CH_3), 3.65 (t, 4H, *J* = 4.8, 2-CH_2N), 3.77 (t, 4H, *J* = 4.8, 2-CH_2O), 8.60 (s, 1H, CH-pyrimidine), 12.23 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 32.4, 44.7, 66.4, 98.4, 153.2, 155.1, 155.6, 160.8; IR: ν 3007,

2970, 2904, 2868, 1672, 1621, 1563 cm^{-1}; MS: *m/z* 235 (M^+); Anal. Calcd for $C_{10}H_{13}N_5O_2$, C, 51.06; H, 5.57; N, 29.77. Found: C, 51.13; H, 5.49; N, 29.80.

1-Methyl-6-(pyrrolidin-1-yl)-1,2-dihydro-3H-pyrazolo[3,4-*d*] pyrimidin-3-one (5b) Reaction time 2 h (A), 2 min (B); yield 69% (A), 86% (B); white powder; mp 230–236°C; ^{1}H NMR: δ 1.90 (m, 4H, 2-CH_2), 3.49 (s, 3H, CH_3), 3.52 (t, 4H, *J* = 6.7 Hz, 2-CH_2N), 8.61 (s, 1H, CH-pyrimidine), 10.91 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 25.3, 32.5, 46.4, 97.7, 153.2, 155.6, 156.5, 159.7; IR: ν 3023, 2970, 2929, 2761, 2667, 2569, 1658, 1617, 1587 cm^{-1}; MS: *m/z* 219 (M^+). Anal. Calcd for $C_{10}H_{13}N_5O$: C, 54.78; H, 5.98; N, 31.94. Found: C, 54.83; H, 6.07; N, 31.89.

1-Methyl-6-(piperidin-1-yl)-1,2-dihydro-3*H*-pyrazolo[3,4-*d*] pyrimidin-3-one (5c) Reaction time 2 h (A), 2 min (B); yield 76% (A), 88% (B); yellow powder; mp 249–251°C; ^{1}H NMR: δ 1.53 (m, 4H, 2-CH_2), 1.63 (m, 2H, CH_2), 3.34 (s, 3H, CH_3), 3.82 (t, 4H, *J* = 5.6 Hz, 2-CH_2N), 8.63 (s, 1H, CH-pyrimidine), 11.04 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 24.6, 25.7, 32.5, 45.1, 97.5, 153.9, 154.1, 156.4, 161.0; IR: ν 3007, 2923, 2851, 2774, 2689, 1682, 1627, 1565 cm^{-1}; MS: *m/z* 233 (M^+). Anal. Calcd for $C_9H_{11}N_5O_2$: C, 56.64; H, 6.48; N, 30.02. Found: C, 56.60; H, 6.42; N, 30.08.

6-Morpholino-2-phenyl-1,2-dihydro-3*H*-pyrazolo[3,4-*d*]pyrimidin-3-one (6a) Reaction time 8 h (A), 6 min (B); yield 77% (A), 90% (B); brown powder; mp 198–201°C; ^{1}H NMR: δ 3.68 (t, 4H, *J* = 4.8 Hz, 2-CH_2N), 3.82 (t, 4H, *J* = 4.4 Hz, 2-CH_2O), 7.19 (t, 2H, phenyl), 7.47 (t, 1H, phenyl), 8.11 (d, 2H, Phenyl), 8.81 (s, 1H, CH-pyrimidine), 11.97 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 44.8, 66.4, 100.0, 119.3, 124.6, 129.5, 139.5, 153.5, 154.8, 155.6, 161.0; IR: ν 3035, 2974, 2900, 2753, 2680, 2573, 1666, 1613, 1596 cm^{-1}; MS: *m/z* 297 (M^+); Anal. Calcd for $C_{15}H_{15}N_5O_2$: C, 60.60; H, 5.09; N, 23.56. Found: C, 60.63; H, 5.02; N, 23.54.

2-Phenyl-6-(pyrrolidin-1-yl)-1,2-dihydro-3*H*-pyrazolo[3,4-*d*] pyrimidin-3-one (6b) Reaction time 8 h (A), 6 min (B); yield 68% (A), 85% (B); white powder; mp 221–224°C; ^{1}H NMR: δ 1.94 (m, 4H, 2-CH_2), 3.56 (t, 4H, *J* = 6.8 Hz, 2-CH_2N), 7.14 (t, 2H, phenyl), 7.45 (t, 1H, phenyl), 8.18 (d, 2H, phenyl), 8.75 (s, 1H, CH-pyrimidine), 11.60 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 25.5, 46.9, 99.2, 118.9, 124.3, 129.4, 139.8, 153.3, 155.2, 155.7, 159.5; IR: ν 3068, 3019, 2970, 2869, 1615, 1539; MS: m/z 281 (M^+). Anal. Calcd for $C_{15}H_{15}N_5O$: C, 64.04; H, 5.37; N, 24.90. Found: C, 64.12; H, 5.30; N, 24.95.

2-Phenyl-6-(piperidin-1-yl)-1,2-dihydro-3*H*-pyrazolo[3,4-*d*] pyrimidin-3-one (6c) Reaction time 8 h (A), 6 min (B); yield 78% (A), 87% (B); yellow powder; mp 198–202°C; ^{1}H NMR: δ 1.55 (m, 4H, 2-CH_2), 1.62 (m, 2H, CH_2), 3.83 (t, 4H, *J* = 5.2 Hz, 2-CH_2N), 7.17 (t, 2H, phenyl), 7.43 (t, 1H, phenyl), 7.95 (d, 2H, phenyl), 8.75 (s, 1H, CH-pyrimidine), 11.73 (br s, 1H, NH, D_2O exchangeable); ^{13}C NMR: δ 24.9, 25.8, 47.2, 102.3, 114.8, 120.7, 125.2, 130.8, 146.6, 152.9, 158.3, 160.4; IR: ν 3051, 2942, 2916, 2851, 1656, 1614 cm^{-1}; MS: *m/z* 295 (M^+). Anal. Calcd for $C_{16}H_{17}N_5O$: C, 65.07, H, 5.80; N, 23.71. Found: C, 65.14; H, 5.81; N, 23.68.

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