Preliminary Communication

Oussama Cherif, Fatma Masmoudi, Fatma Allouche, Fakher Chabchoub* and Mohamed Trigui

Synthesis, antibacterial, and antifungal activities of new pyrimidinone derivatives

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Abstract: An efficient synthesis of new pyrrolopyrimidinones **3a-d** and isoxazolopyrimidinones **4a-c** from the respective aminocyanopyrroles **1a-d** and aminocyanoisoxazoles **2a-c** is presented. The synthesized compounds were screened for antimicrobial activity against a panel of bacteria and fungi. Compound **4c** exhibits remarkable activity against a broad spectrum of Gram-positive and Gram-negative bacteria and pathogenic fungi.

Keywords: antimicrobial activity; isoxazolopyrimidinones; pyrrolopyrimidinones.

Pyrroles and isoxazoles [1–4] have received intensive research interests because of their biological activities and have found a wide range of applications in pharmaceutical and agrochemical fields [5–8]. In continuation of our previous study on the synthesis and biological activity of heterocyclic compounds [9], we now report the preparation of a series of pyrrolopyrimidinones **3a-d** and isoxazolopyrimidinones **4a-c** starting with readily available pyrroles **1a-d** and isoxazoles **2a-c** [10, 11]. Compounds **3a-d** and **4a-c** were synthesized by heating the respective substrates **1a-d** and **2a-c** under reflux in formic acid in the presence of a catalytic amount of sulfuric acid. The yields ranged from 57% to 81% (Scheme 1). Mechanistically, the reaction may involve the partial hydrolysis of the cyano group to give an amide and the formylation of the amino group to give a formamide followed by intramolecular condensation of these two functionalities, as shown.

All compounds were screened for their antibacterial and antifungal activities against a number of reference test organisms, including five Gram-positive, three Gram-negative, and eight plant pathogenic fungi. The study of antimicrobial activities showed that compounds **1–3** and **4a,b** exhibit very low activities. A notable exception is compound **4c**, which is active against a broad spectrum of Gram-positive and Gram-negative bacteria (Table 1). The same compound **4c** shows also high antifungal activity (Table 2).

Experimental

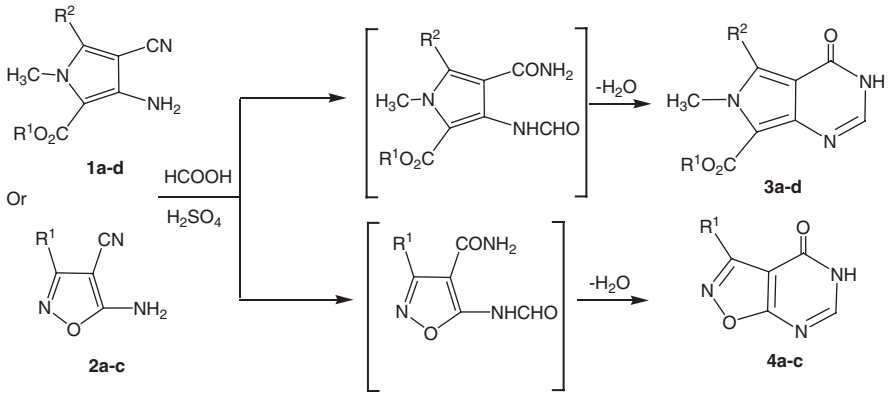
Melting points were measured on an electrothermal apparatus. Progress of the reactions was monitored by thin-layer chromatography (TLC) using aluminum sheets with silica gel 60 F254 from Merck. Infrared spectra (IR) were recorded on a Perkin-Elmer Paragon FT-IR spectrometer using KBr pellets. Unless stated otherwise, ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker spectrometer (^{1}H at 400 MHz, ^{13}C at 100 MHz). Elemental analyses were performed on a Carlo Erba 1106 apparatus. Substrates **1a-d** and **2a-c** were synthesized as previously reported [10, 11].

General procedure for the synthesis of compounds 3a-d and 4a-c

3-Amino-4-cyano-substituted pyrrole **1** or 5-amino-4-cyano-substituted isoxazole **2** (10 mmol) was added portionwise for 2 h to a mildly refluxing mixture of formic acid (20 mL) and concentrated sulfuric acid (1.2 mL). After an additional 30 min, the solution was cooled to 0°C and poured onto crushed ice. The resulting precipitate was collected by filtration, washed with water, dried, and crystallized from ethanol to give a pyrrolopyrimidinone **3** or an isoxazolopyrimidinone **4**.

Methyl 5,6-dimethyl-4-oxo-4,6-dihydro-3*H*-pyrrolo[3,4-*d*] pyrimidine-7-carboxylate (3a) Yield 81%; yellow solid; mp 231–233°C; IR (cm^{-1}): ν 1596, 1692, 3187; ^{1}H NMR: δ 8.29 (s, 1H, NH), 7.89 (s, 1H, H-2), 4.01 (s, 3H, CH_3-7), 2.76 (s, 3H, CH_3-6), 2.19 (s, 3H, CH_3-5); ^{13}C NMR: δ 159.3 (C-4), 157.7 (CO_2Me), 145.2 (C-2), 143.6 (C-5), 133.9 (C-7a),

***Corresponding author: Fakher Chabchoub,** Laboratory of Applied Chemistry Heterocycles, Fats and Polymers, Faculty of Sciences of Sfax, University of Sfax, Sfax 3018, Tunisia,
e-mail: fakher.chabchoub@yahoo.fr
Oussama Cherif and Fatma Allouche: Laboratory of Applied Chemistry Heterocycles, Fats and Polymers, Faculty of Sciences of Sfax, University of Sfax, Sfax 3018, Tunisia
Fatma Masmoudi and Mohamed Trigui: Biopesticides Team (LPIP), Center of Biotechnology of Sfax, University of Sfax, PO Box 1177, Sfax 3018, Tunisia



Scheme 1

Table 1 Antibacterial activities of compound **4c** *in vitro*.

		Bacterial strains							
		Gram-positive bacteria					Gram-negative bacteria		
		Bc	**Sa**	**Ef**	**Ml**	**Lm**	**Ec**	**Se**	**Kp**
Compound **4c**	IZ[a]	18	10	13	09	12	11	12	15
	MIC[b]	0.01	0.043	0.085	0.085	0.687	0.085	0.172	0.085
Gentamicin[c]	IZ	20	20	20	18	20	25	25	22
	MIC	0.004	0.004	0.004	0.004	0.001	0.002	0.002	0.002
DMSO 20%	IZ[d]	–	–	–	–	–	–	–	–

Bacterial strains: Bc, *Bacillus cereus* ATCC14579; Sa, *Staphylococcus aureus* ATCC25923; Ef, *Enterococcus faecalis* ATCC 29212; Ml, *Micrococcus luteus* ATCC 1880; Lm, *Listeria monocytogenes* (Fl 2132); Ec, *Escherichia Coli* ATCC 25922; Se, *Salmonella enteritidis* (food isolate); Kp, *Klebsiella pneumonia* CIP 32147.

[a]Diameter of the inhibition zone (IZ) in mm of a compound, including the diameter of the disc (6 mm).

[b]Minimum inhibitory concentration in mg/mL.

[c]Gentamicin was used as a standard antibiotic at 15 μg/mL.

[d]Activity not detected.

Table 2 Antifungal activities of compound **4c**.

Compounds		**Fungal strains**							
		Rn	**Fo**	**Aa**	**Pp**	**Fc**	**Bc**	**Fg**	**Af**
4c	IZ[a]	11	10	13	12	13	15	10	19
	MIC[b]	0.687	0.172	0.687	0.343	0.172	0.687	0.343	5.5
Amphotericin[c]	IZ	10	12	12	18	14	12	14	10
	MIC	0.343	0.042	0.172	0.312	0.085	0.172	0.343	0.172
DMSO 20%	IZ[d]	–	–	–	–	–	–	–	–

Fungal strains: Rn, *Rhizopus nigricans* (LPAP26); Fo, *Fusarium oxysporum* CTM10402; Aa, *Alternaria alternata* CTM 10230; Pp, *Pythium aphanidermatum* (LPAP32); Fc, *Fusarium culmorum* ISPAVE 21W; Bc, *Botrytis cinerea* (LPAP34); Fg, *Fusarium graminearum* ISPAVE 271; Af, *Aspergillus flavus* (food isolate).

[a]Diameter of the inhibition zone of a compound, including the diameter of the disc (6 mm).

[b]Minimal inhibitory concentration in mg/mL.

[c]Amphotericin B was used as antifungal standard at 20 μg/mL.

[d]Activity not detected.

114.1 (C, C-7), 106.6 (C-4a), 31.8 (CH_3-7), 13.3 (CH_3-6), 10.6 (CH_3-5). Anal. Calcd for $C_{10}H_{11}N_3O_3$: C, 54.29; H, 5.01; N, 19. Found: C, 53.80; H, 5.20; N, 18.33.

Ethyl 5,6-dimethyl-4-oxo-4,6-dihydro-3*H*-pyrrolo[3,4-*d*]pyrimidine-7-carboxylate (3b) Yield 71%; yellow solid; mp 243–245°C; IR (cm^{-1}): ν 1597, 1691, 3173; ^{1}H NMR: δ 8.24 (s, 1H, NH), 8.12 (s, 1H, H-2), 4.43 (q, 2H, ^3J = 7.5 Hz, CH_2), 3.91 (s, 3H, CH_3-6), 2.61 (s, 3H, CH_3-5), 1.33 (t, 3H, ^3J = 7.5 Hz, CH_3-7); ^{13}C NMR: δ 160.1 (C-4), 159.1 (CO_2Et), 146.2 (C-2), 144.9 (C-5), 135.3 (C-7a), 115.4 (C-7), 107.0 (C-4a), 59.9 (CH_2), 32.9 (CH_3-6), 14.4 (CH_3-7), 11.0 (CH_3-5). Anal. Calcd for $C_{11}H_{13}N_3O_3$: C, 56.16; H, 5.57; N, 17.86. Found: C, 55.66; H, 6.02; N, 17.38.

Methyl 5-ethyl-6-methyl-4-oxo-4,6-dihydro-3*H*-pyrrolo[3,4-*d*]pyrimidine-7-carboxylate (3c) Yield 73%; yellow solid; mp 247–250°C; IR (cm^{-1}): ν 1597, 1680, 3187; ^{1}H NMR: δ 8.32 (s, 1H, NH), 8.11 (s, 1H, H-2), 4.09 (s, 3H, CH_3-7), 4.01 (s, 3H, CH_3-6), 3.23 (q, 2H, ^3J = 7.5 Hz, CH_2), 1.29 (t, 3H, ^3J = 7.5 Hz, CH_3-5); ^{13}C NMR: δ 160.7 (C-4), 158.2 (CO_2Me), 145.8 (C-2), 141.2 (C-5), 139.5 (C-7a), 111.3 (C-7), 106.0 (C-4a), 51.0 (CH_3-7), 32.9 (CH_3-6), 17.5 (CH_3-5), 13.1 (CH_2). Anal. Calcd for $C_{11}H_{13}N_3O_3$: C, 56.16 H, 5.57; N, 17.86 . Found: C, 55.04; H, 5.83; N, 17.29.

Methyl 6-methyl-4-oxo-5-phenyl-4,6-dihydro-3*H*-pyrrolo[3,4-*d*]pyrimidine-7carboxylate (3d) Yield 65%; yellow solid; mp 232–235°C; IR (cm^{-1}): ν 1600, 1693, 3296; ^{1}H NMR: δ 8.34 (s, 1H, NH), 8.13 (s, 1H, H-2), 7.46–7.56 (m, 5H, H-arom), 4.14 (s, 3H, CH_3-7), 4.01 (s , 3H, CH_3-6); ^{13}C NMR: δ 161.1 (C-4), 157.7 (CO_2Me), 145.4 (C-2), 141.4 (C-5), 127.9–136.9 (C-arom), 115.3 (C-7a), 114.4 (C-7), 107.5 (C-4a), 51.1 (CH_3-7), 35.0 (CH_3-6). Anal. Calcd for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83. Found: C, 62.76; H, 4.64; N, 14.29.

3-Methylisoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one (4a) Yield 66%; white solid; mp 232–235°C; IR (cm^{-1}): ν 1567, 1707, 3182; ^{1}H NMR: δ 12.74 (s, 1H, NH), 8.28 (s, 1H, H-6), 2.46 (s, 3H, CH_3); ^{13}C NMR: δ 175.10 (C-7a), 157.7 (C-4), 157.2 (C-3), 152.2 (C-6), 101.5 (C-3a), 10.9 (CH_3). Anal. Calcd for $C_6H_5N_3O_2$: C, 47.69; H, 3.33; N, 27.81. Found: C, 46.83; H, 4.16; N, 27.05.

3-ethylisoxazolo[5,4-d]pyrimidin-4(5*H*)-one (4b) Yield 61%; white solid; mp 185–188°C; IR (cm^{-1}): ν 1567, 1702, 3188; ^{1}H NMR: δ 12.21 (s, 1H, NH), 8.24 (s, 1H, H-6), 3.03 (q, 2H, ^3J = 7.7 Hz, CH_2), 1.45 (t, 3H, ^3J = 7.7 Hz, CH_3); ^{13}C NMR: δ 175.9 (C-7a), 158.9 (C-4); 157.8 (C-3), 154.2 (C-6), 102.7 (C-3a), 15.9 (CH_2), 10.6 (CH_3). Anal. Calcd for $C_7H_7N_3O_2$: C, 50.91; H, 4.27; N, 25.44. Found: C, 50.95; H, 4.37; N, 25.37.

3-Phenylisoxazolo[5,4-*d*]pyrimidin-4(5*H*)-one (4c) Yield 57%; white solid; mp 216–218°C; IR (cm^{-1}): ν 1559, 1692, 3190; ^{1}H NMR: δ 10.58 (s, 1H, NH), 8.12 (s, 1H , H-6), 7.19–8.24 (m, 5H, H-$_{arom}$); ^{13}C NMR: δ 170.5 (C-7a), 161.2 (C-4) , 160.2 (C-3), 144.7 (C-6), 123.4–130.7 (C-C_{arom}), 115.4 (C-3a). Anal. Calcd for $C_{11}H_7N_3O_2$: C, 61.97; H, 3.31; N, 19.71. Found: C, 61.32; H, 3.68; N, 18.69.

Antimicrobial activity

Eight bacterial and eight fungal strains were used. The tested pathogenic bacteria were *Bacillus cereus* ATCC 14579 (*Bc*), *Staphylococcus aureus* ATCC 25923 (*Sa*), *Enterococcus faecalis* ATCC 29212 (*Ef*), *Micrococcus luteus* ATCC 1880 (*Ml*), *Escherichia coli* ATCC 25922 (*Ec*), *Klebsiella pneumoniae* ATCC 10031 (*Kp*), *Salmonella enteritidis* (food isolate 824) (*Se*), and *Listeria monocytogenes* (food isolate 2132) (*Lm*). The fungi tested were *Rhizopus nigricans* (LPAP26) (*Rn*), *Fusarium oxysporum* CTM10402 (*Fo*), *Alternaria alternata* CTM 10230 (*Aa*), *Pythium aphanidermatum* (LPAP32) (*Pp*), *Fusarium culmorum* ISPAVE 21W (*Fc*), *Botrytis cinerea* (LPAP34) (*Bc*), *Fusarium graminearum* ISPAVE 271 (*Fg*), and *Aspergillus flavus* (food isolate) (*Af*). The bacterial strains were cultivated in Mueller-Hinton (MH) agar (Oxoid Ltd., UK) at 37°C except for *Bacillus* species, which were incubated at 30°C. The fungi were cultured on potato dextrose agar (PDA) medium and incubated at 28°C. Working cultures were prepared by inoculating a loopful of each test bacteria in 3 mL of MH broth (Oxoid Ltd., UK) and were incubated at 37°C for 12 h. For the test, final inoculum concentrations of 10^7 CFU/mL of bacteria were used. Fungal spore suspensions were collected from the surface of such fungal colonies by gently scraping with a loop and suspended in 10 mL potato dextrose broth. This suspension was mixed vigorously by vortexing for 15–20 min. The spore suspension stock was diluted to obtain a concentration of 10^6 spores/mL (measured by Malassez blade). Antibacterial and antifungal tests were performed by disc diffusion method [12] and broth microdilution assay using sterile MH media (Bio-Rad, France) for bacterial strains PDA (Bio-Rad, France) for antifungal tests. Freshly prepared cell suspension (100 µL) adjusted to 10^7 CFU/mL for bacteria and 10^6 spores/mL for fungus were inoculated onto the surface of agar plates. Thereafter, discs with 6 mm in diameter were punched in the inoculated agar medium with sterile Pasteur pipettes, and compounds were added to each disc. Gentamicin (10 µg/disc) was used as a positive control for bacteria, whereas amphotericin B (20 µg/disc) was used as a positive control for fungal strains. The plate was allowed to stand for 2 h at 4°C to permit the diffusion of the compounds followed by incubation at 37°C for 24 h for bacterial strains and 72 h for fungi at 28°C. The antibacterial activity was evaluated by measuring the zones of inhibition (clear zone around the disc) against the tested microorganisms. All tests were repeated three times. Minimum inhibitory concentrations (MICs) of compounds were determined in sterile 96-well microplates with a final volume in each microplate well of 200 µL [13]. A twofold serial dilution of the compound was prepared in the microplate wells over the range 0.01–5.5 mg/mL. To each test well was added 10 µL cell suspension to final inoculum concentrations of 10^6 CFU/mL for bacteria and 10^5 spores/mL for fungi. The plates were then covered with the sterile plate covers and incubated at 37°C for 24 h for bacterial strains and 72 h for fungi at 28°C. The MIC was defined as the lowest concentration of the compound at which the microorganism does not demonstrate visible growth after incubation. As an indicator of microorganism growth, 25 µL of 0.5 mg/mL *p*-iodonitrotetrazolium chloride, dissolved in sterile water, was added to the wells and incubated at 37°C for 30 min. The lowest concentration of compound showing no growth was taken as its MIC.

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