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ORIGINAL PAPER

Synthesis and antibacterial activity of some new derivatives of pyrazole

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Abstract In this study, we report the synthesis and antibacterial activity of a new series of 5-amido-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolecarbonitriles. Our results show that all compounds exhibit antimicrobial activities against methicillin susceptible *Staphylococcus aureus* and methicillin resistant *S. aureus* with MIC values of 25.1 and 91.0 μM.

Keywords Pyrazoles · Antibacterial agents · *Staphylococcus aureus* · MIC

Introduction

Pyrazoles are one of the oldest class of bioactive compounds which are widely used as anti-inflammatory (Bekhit et al. 2008; Bekhit and Abdel-Aziem 2004; Ochi et al. 1999), antipyretic (Souza et al. 2002; Menozzi et al. 1993), antiarrhythmic (Bruno et al. 1997), anticonvulsant (Satoh et al. 1979; Batulin 1968), monoamine oxidase inhibiting (Chimenti et al.2006a, b, 2007; antibacterial (Bildirici et al. 2007; Tanitame et al. 2004; Berghot and Moawad 2003; Wick et al. 1973) agents. The clinical significance of this class of compounds has stimulated the synthesis of new lead compounds retaining the 'core' pyrazole chromophore.

Pyrazoles are produced synthetically through the reaction of α,β-unsaturated aldehydes with hydrazine and subsequent dehydregenation. 3- or 5-aminopyrazoles are prepared *via* a dihydro precursor which is formed by addition of hydrazine to acrylonitrile (Dorn and Zubek 1971). The pyrazole ring also can be formed by the reaction of hydrazine with propargyl aldehyde (Pérez et al. 1996).

Continuing our study on the activity of pyrazoles against bacteria (Sadeghian et al. 2009), in this work, we wish to report the synthesis of some new derivatives of pyrazole which show moderate to strong antimicrobial activities against gram positive bacteria.

Results and discussion

Chemistry

The precursor 5-amino-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolecarbonitrile **1** were prepared from the reaction of 1-(2,4-dinitrophenyl)hydrazine with 2-(ethoxymethylene) malononitrile in ethanol under reflux condition (Kreutzberger and Burgwitz 1980). The new 5-amino-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolecarbonitrile carboxylates **3a–j** were synthesized from the reaction of compound **1** and corresponding acid chlorides **2a–j** which were either purchased or prepared (**3g, 3h** and **3j**) by reaction of thionyl chloride and corresponding carboxylic acids (Villani and King 1963). All desired amides were synthesized by the action of the acid chlorides with compound **1** in dry pyridine. (Scheme 1).

Biological evaluations

The compound **1** and synthetic compounds **3a–j** were screened for the antibacterial activity against *Escherichia*

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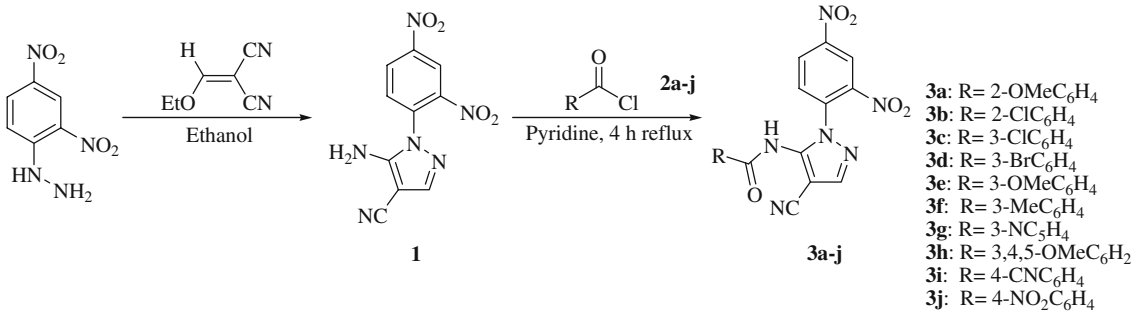


Scheme 1 Structural assignments of compounds **3a–j** was based upon the spectral and microanalytical (C, H and N) data

coli HB101 (BA-7601C), Staphylococcuse aureus pathogens [methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA—ATCC 1112)], *Pseudomonas aeruginosa (PTCC 1431), and Bacillus subtilis (PTCC 1365)*. The minimum inhibitory concentrations (MIC) of **3a–j** were determined in dilution test tube method, which had been introduced by NCCLS (National Committee for Clinical Laboratory Standards) (Finegold and Garrod 1995). For broth dilution methods, in which decreasing concentrations of the antimicrobial agents must be tested, usually prepared in serial twofold dilution of a broth medium is placed in tubes which will support the growth of the test microorganism (10^4 CFU mL^{-1}). After sufficient incubation (18 h), the tubes are examined for turbidity, indicating growth of the microorganism. The organism will grow in the tube that does not contain enough antimicrobial agents to inhibit growth. For further confidence, the samples were cultured onto Petri dishes containing *Mueller Hinton agar* (18 h at 37°C). The lowest concentration of the antibacterial agent that prevents growth of the test organism, as detected by lack of visual turbidity (matching the negative growth control), is designated the minimum inhibitory concentration (MIC). A serial dilution of tested compounds (final concentration of 400–0.4 μg mL^{-1}), were added to the test bacteria in Mueller–Hinton broth and were incubated at 37°C for 18 h. Growth was presented in the medium control and was absent from the inoculum control (Phillips et al. 1978). The results of these studies are given in Table 1.

As it can be seen from the data in Table 1, these compounds are only effective against gram positive bacteria. Compound **1** was not effective at all unlike the other synthesized compounds **3a–j**. Compounds **3i** and **3j** which have *para* substitute in R group, showed the best inhibitory effects against methicillin resistant *S. aureus* (MRSA) and methicillin susceptible *S. aureus* (MSSA—ATCC 1112) (MIC **3i** = 29.4 μg mL^{-1}; **3j** = 25.1 μg mL^{-1} for the both strains). All the other compounds were found to exhibit moderate activities against the mentioned organisms. These results clearly demonstrate when compound **1** converted to corresponding amides, it exhibited powerful antibacterial activity. These results are compared with MIC values of Cephalexin (72 and 4.6 μg mL^{-1}), Cloxacillin (94 and 13.7 μg mL^{-1}) and Erythromycin (32 and 32 μg mL^{-1}). As it can be seen from the data in Table 1, compound **3j** shows the more inhibitory activity against methicillin susceptible *S. aureus* (MSSA—ATCC 1112) than Cephalexin, Cloxacillin and Erythromycin.

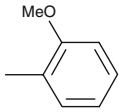
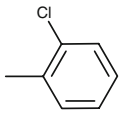
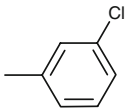
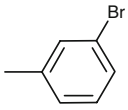
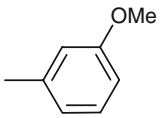
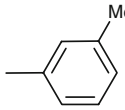
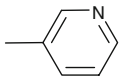
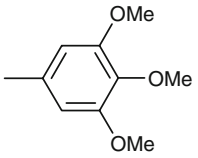
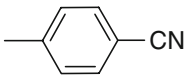
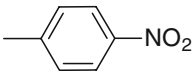
In summary, we have synthesized some new derivatives of pyrazoles as *S. aureus* growth inhibitors which can be used in the bigger scenario such as in drug design or development of antimicrobial therapeutics and have also shown the important role of the carboxylate moiety in the inhibitory activities of compounds **3a–j**.

Experimental

Melting points were recorded on an Electrothermal type 9100 melting point apparatus. The IR spectra were obtained on a 4300 Shimadzu spectrometer and only noteworthy absorptions are listed. The ^1H NMR (100 MHz) spectra were recorded on a Bruker AC 100 spectrometer. Chemical shifts are reported in ppm downfield from TMS as internal standard; coupling constance J are given in Hertz. The mass spectra were scanned on a Varian. Mat CH-7 at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyzer.

All of the chemicals were purchased from Sigma, Fluka, Calbiochem and Merck Co. The microorganisms *S. aureus* ATCC 1112 were purchased from Pasteur Institute of Iran and *S. aureus* (methicillin resistant) was isolated from different specimens which were referred to the Microbiological Laboratory of Ghaem Hospital of Medical University of Mashhad,Iran and its methicillin resistance was tested according to the NCCLS guidelines (Finegold and Garrod 1995).

Table 1 Minimum inhibitory concentration (μg mL^{-1}) of the synthetic compounds **3a–j**

Compound	R	MRSA	MSSA	*E. coli*
1	–	–	–	–
3a		63.8	63.8	>400
3b		91.0	70.5	>400
3c		46.0	46.0	>400
3d		70.3	60.8	>400
3e		39.3	35.3	>400
3f		80.5	80.5	>400
3g		33.0	33.0	>400
3h		45.3	35.9	>400
3i		29.4	29.4	>400
3j		25.1	25.1	>400
Erythromycin	–	32.0	32.0	15
Cloxacillin	–	94.0	13.7	20
Cephalexin	–	72.0	4.6	17

General procedure for preparation of the 5-amino-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolecarbonitrile **3a–j**

A mixture of ethoxymethylenmalononitril (12.2 g, 100 mmol) and 2,4-dinitrophenylhydrazine (19.8 g, 100 mmol) in ethanol (200 mL) were heated under reflux for 4 h. After cooling, the crystals of product were appeared. Then the crystals were separated and washed with ethanol and dried at 70°C to give compound **1** (18.4 g, 67%, mp 218°C).

To a stirred solution of **1** (2.74 g, 10 mmol) in dry pyridine (20 mL) was added acid chlorides (12 mmol) dropwise at room temperature. After refluxing for 4 h, the pyridine was evaporated under reduced pressure. The residue was treated with 5% sodium carbonate (2 × 50 mL) and extracted with dichloromethane (2 × 30 mL). The organic extract was dried with anhydrous sodium sulfate, concentrated under reduced pressure and crystallized to provide the pure desired compound **3a–j**.

N_1-[4-Cyano-1-(2,4-dinitrophenil)-1H-5-pyrazolil]-2-methoxybenzamid ***(3a)***

Light yellow crystals (ethanol). Yield: 63%; mp 203-205°C. ^{1}H NMR ($CDCl_3$): δ 4.00 (s, 3H), 7.09–7.67 (m, 4H), 7.95 (d, 1H, $J = 8.8$ Hz), 8.08 (s, 1H), 8.80 (dd, 1H, $J = 8.8$ Hz, $J' = 2.4$ Hz), 8.98 (d, 1H, $J = 2.4$ Hz), 9.70 (br s, 1H); IR: 1,705 (C=O) and 3,243 (NH) cm^{-1}. MS (70 eV): $m/z = 408$ (M^+). Found: C, 52.79; H, 2.91; N, 20.46. $C_{18}H_{12}N_6O_6$ (408.33) requires: C, 52.95; H, 2.96; N, 20.58%.

N_1-[4-Cyano-1-(2,4-dinitrophenil)-1H-5-pyrazolil]-2-chlorobenzamid ***(3b)***

Light yellow crystals (ethanol). Yield: 67%; mp 298–300°C. ^{1}H NMR ($CDCl_3$): δ 7.25–7.75 (m, 4H), 8.02 (d, 1H, $J = 8.8$ Hz), 8.06 (s, 1H), 8.4 (br s, 1H), 8.65 (dd, 1H, $J = 8.8$ Hz, $J' = 2.4$ Hz), 8.95 (d, 1H, $J = 2.4$ Hz); IR: 1,704 (C=O) and 3,228 (NH) cm^{-1}. MS (70 eV): $m/z = 414$ (M^++2). Found: C, 49.13; H, 2.13; N, 20.19. $C_{17}H_9N_6O_5Cl$ (412.75) requires: C, 49.47; H, 2.20; N, 20.36%.

N_1-[4-Cyano-1-(2,4-dinitrophenil)-1H-5-pyrazolil]-3-chlorobenzamid ***(3c)***

White crystals (ethanol). Yield: 69%; mp 202–205°C. ^{1}H NMR ($CDCl_3$): δ 7.45–7.91 (m, 4H), 8.22 (d, 1H, $J = 8.8$ Hz), 8.31 (s, 1H), 8.77 (dd, 1H, $J = 8.8$ Hz, $J = 2.4$ Hz), 8.93 (d, 1H, $J = 2.4$ Hz), 10.53 (br s, 1H); IR: 1,705 (C=O) and 3,217 (NH) cm^{-1}. MS (70 eV): *m/*

$z = 414$ (M^++2). Found: C, 49.39; H, 2.17; N, 20.31. $C_{17}H_9N_6O_5Cl$ (412.75) requires: C, 49.47; H, 2.20; N, 20.36%.

N_1-[4-Cyano-1-(2,4-dinitrophenyl)-1H-5-pyrazolyl]-3-bromobenzamide ***(3d)***

Light yellow crystals (ethanol). Yield: 73%; mp 214–216°C. ^{1}H NMR ($CDCl_3$): δ 7.33–7.79 (m, 4H), 8.24 (d, 1H, $J = 8.8$ Hz), 8.30 (s, 1H), 8.79 (dd, 1H, $J = 8.8$ Hz, $J = 2.4$ Hz), 8.91 (d, 1H, $J = 2.4$ Hz), 10.11 (br s, 1H); IR: 1,705 (C=O) and 3,217 (NH) cm^{-1}. MS (70 eV): $m/z = 459$ (M^++2). Found: C, 44.23; H, 1.83; N, 18.12. $C_{17}H_9N_6O_5Br$ (457.20) requires: C, 44.66; H, 1.98; N, 18.38%.

N_1-[4-Cyano-1-(2,4-dinitrophenil)-1H-5-pyrazolil]-3-methoxybenzamid ***(3e)***

Light yellow crystals (ethanol). Yield: 78%; mp 206–208°C.^{1}H NMR ($CDCl_3$): δ 3.81 (s, 3H), 7.09–7.51 (m, 4H), 7.95 (d, 1H, $J = 8.8$ Hz), 8.14 (s, 1H), 8.56 (dd, 1H, $J = 8.8$ Hz, $J' = 2.4$ Hz), 8.79 (d, 1H, $J = 2.4$ Hz), 9.30 (br s, 1H);. IR: 1,653 (C=O) and 3,230 (NH) cm^{-1}. MS (70 eV): $m/z = 408$ (M^+). Found: C, 52.58; H, 2.81; N, 20.19. $C_{18}H_{12}N_6O_6$ (408.33) requires: C, 52.95; H, 2.96; N, 20.58%.

N_1-[4-Cyano-1-(2,4-dinitrophenil)-1H-5-pyrazolil]-3-methylbenzamid ***(3f)***

White crystals (ethanol). Yield: 81%; mp 201–203°C. ^{1}H NMR ($CDCl_3$): δ 2.38 (s, 3H), 7.42–7.58 (m, 4H), 7.97 (d, 1H, $J = 8.8$ Hz), 8.14 (s, 1H), 8.56 (dd, 1H, $J = 8.8$ Hz, $J' = 2.4$ Hz), 8.79 (d, 1H, $J = 2.4$ Hz), 9.30 (br s, 1H); IR: 1,704 (C=O) and 3,232 (NH) cm^{-1}. MS (70 eV): $m/z = 392$ (M^+). Found: C, 55.03; H, 3.11; N, 21.34. $C_{18}H_{12}N_6O_5$ (392.33) requires: C, 55.11; H, 3.08; N, 21.42%.

N_3-[4-Cyano-1-(2,4-dinitrophenil)-1H-5-pyrazolil]-nicotineamid ***(3g)***

Light yellow crystals (ethanol). Yield: 73%; mp 103–106°C. ^{1}H NMR ($CDCl_3$): δ 7.35–7.55 (m, 1H), 8.12 (d, 1H, $J = 8.8$ Hz), 8.21 (s, 1H), 8.23 (br s, 1H), 8.63 (dd, 1H, $J = 8.8$ Hz, $J' = 2.4$ Hz), 8.65–8.81 (m, 3H), 8.91 (d, 1H, $J = 2.4$ Hz); IR: 1,705 (C=O) and 3,231 (NH) cm^{-1}. MS (70 eV): $m/z = 379$ (M^+). Found: C, 50.39; H, 2.25; N, 25.61. $C_{16}H_9N_7O_5$ (379.29) requires: C, 50.67; H, 2.39; N, 25.85%.

N_1-[4-Cyano-1-(2,4-dinitrophenil)-1H-5-pyrazolil]-3,4,5-methoxybenzamid ***(3h)***

Light yellow crystal (ethanol). Yield: 66%; mp 140–142°C. ^{1}H NMR ($CDCl_3$): δ 3.78 (s, 3H), 3.95 (s, 6H), 7.09 (s, 2H), 7.97 (d, 1H, $J = 8.8$ Hz), 8.14 (s, 1H), 8.56 (dd, 1H, $J = 8.8$ Hz, $J' = 2.4$ Hz), 8.79 (d, 1H, $J = 2.4$ Hz), 9.30 (br s, 1H); IR: 1,705 (C=O) and 3,230 (NH) cm^{-1}. MS (70 eV): $m/z = 468$ (M^+). Found: C, 51.12; H, 3.36; N, 17.78. $C_{20}H_{16}N_6O_8$ (468.38) requires: C, 51.29; H, 3.44; N, 17.94%.

N1-[4-cyano-1-(2,4-dinitrophenyl)-1H-5-pyrazolyl]-4-cyanobenzamide ***(3i)***

White crystals (ethanol). Yield: 75%; mp 276–278°C. ^{1}H NMR ($CDCl_3$): δ 7.55 (d, 2H, $J = 8.3$ Hz), 7.93 (d, 2H, $J = 8.3$ Hz), 8.20 (d, 1H, $J = 8.8$ Hz), 8.29 (s, 1H), 8.79 (dd, 1H, $J = 8.8$ Hz, $J = 2.4$ Hz), 8.91 (d, 1H, $J = 2.4$ Hz), 9.81 (br s, 1H); IR: 1,705 (C=O) and 3,217 (NH) cm^{-1}. MS (70 eV): $m/z = 403$ (M^+). Found: C, 53.23; H, 2.17; N, 24.11. $C_{18}H_9N_7O_5$ (403.31) requires: C, 53.61; H, 2.25; N, 24.31%.

N_1-[4-cyano-1-(2,4-dinitrophenyl)-1H-5-pyrazolyl]-4-nitrobenzamide ***(3j)***

Light yellow crystals (ethanol). Yield: 70%; mp 291–293°C. ^{1}H NMR ($CDCl_3$): δ 7.65 (d, 2H, $J = 8.0$ Hz), 7.74 (d, 2H, $J = 8.0$ Hz), 8.22 (d, 1H, $J = 8.8$ Hz), 8.28 (s, 1H), 8.81 (dd, 1H, $J = 8.8$ Hz, $J = 2.4$ Hz), 8.89 (d, 1H, $J = 2.4$ Hz), 10.21 (br s, 1H); IR: 1,705 (C=O) and 3,217 (NH) cm^{-1}. MS (70 eV): $m/z = 423$ (M^+). Found: C, 47.90; H, 2.07; N, 22.89. $C_{17}H_9N_7O_7$ (423.30) requires: C, 48.24; H, 2.14; N, 23.16%.

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