Synthesis and biological evaluation of novel pyrazole derivatives as antibacterial agents

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The synthesis of a novel series of 5-[(X-benzoylamino)]-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolecarboxamide derivatives is described. Their antibacterial activity (MIC) against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* (MRSA) were determined. The results revealed that two of the compounds, X = 4-MeO and X = 4-NO_2, displayed greater antibacterial activity against MRSA than did cephalexin, cloxacillin and erythromycin.

Keywords: antibacterial agents, pyrazoles, *Staphylococcus aureus*, MRSA, MIC

Resistance to antimicrobial agents is recognised as a major global public health problem, so that the discovery of new antibacterial and antifungal compounds has become increasingly critical in fighting infectious disease.

In continuation of our previous studies on the activity of trisubstituted pyrazoles against bacteria,[1,2] we report the synthesis of some analogues of those compounds which show much improved antimicrobial activities against gram positive bacteria.

Results and discussion

We have reported[1,2] the synthesis and biological evaluation of a series of *N*-acylated derivatives of 5-amino-1-(2,4-dinitrophenyl)-1H-4-pyrazolecarbonitrile **1** (see Scheme 1). Here we report on the synthesis and testing of the corresponding *N*-acyl derivatives of the amido compound **2**.

Compound **1**, which was readily obtained[1,2] from the reaction of 2,4-dinitrophenylhydrazine with 2-(ethoxymethylene) malononitrile in ethanol under reflux using a literature method,[3] was smoothly hydrolysed by sulfuric acid at room temperature[4] to afford 5-amino-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolecarboxamide **2**. The new 5-acylamino-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolecarboxamides **4a–h** were synthesised from the reaction of compound **2** and the corresponding acid chlorides **3a–h** in dry pyridine (Scheme 1).

Structural assignments of compounds **4a–h** were based upon their spectral and microanalytical (C, H and N) data.

The synthetic compounds **1**, **2** and **4a–h** were screened for antibacterial activity against *Escherichia coli HB101 (BA-7601C), Staphylococcus aureus* pathogens (methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA - ATCC 1112)), *Pseudomonas aeruginosa (PTCC 1431),* and *Bacillus subtilis (PTCC 1365)*. Experimental details of the tests can be found in our earlier study.[1]

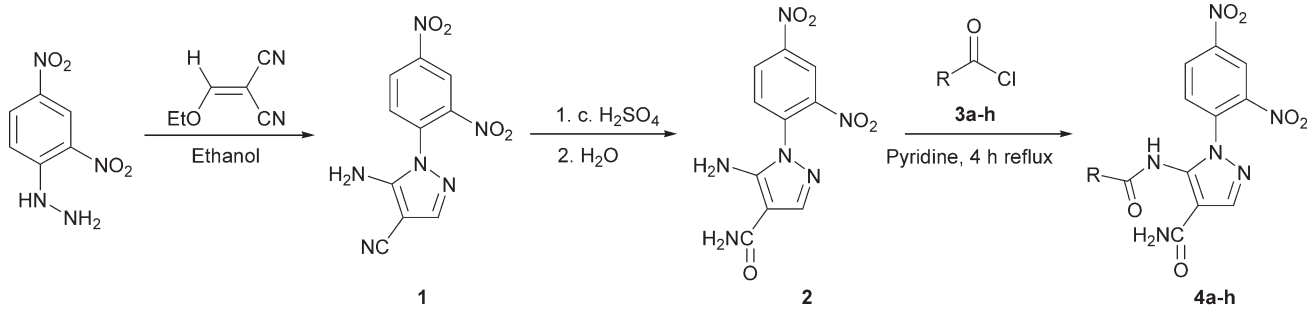
These compounds were effective only against gram positive bacteria, and their activities against MRSA and MSSA are shown in Table 1. Synthetic intermediates **1** and **2** were not effective at all. Compounds **4a–d** and **4f** which have *para* substitutents in the R group, showed the best inhibitory effects against both MRSA and MSSA. **4e**, **4g** and **4h** were found to exhibit moderate antibacterial activities. Gratifyingly, these results clearly show that when the cyano group in compound **1** is hydrolysed to the corresponding amide **2**, and the latter converted to new compounds **4a–h,** they exhibited powerful antibacterial activity in comparison with previous pyrazole derivatives which we have synthesised.[1,2] As the data in Table 1 show, compound **4f** shows greater inhibitory activity against MRSA and MSSA than did the well known antibacterial agents cephalexin, cloxacillin and erythromycin.

To summarise, we have synthesised some novel trisubstituted pyrazoles and shown them to be very effective *S. aureus* growth inhibitors. Such compounds would appear to offer a suitable template for the design of more powerful antibacterial agents and further studies are under way to this end in our laboratory.

Experimental

Melting points were recorded on an Electrothermal type-9100 melting-point apparatus. The IR spectra (as KBr discs) were obtained on a Tensor 27 spectrometer. ^{1}H NMR spectra were recorded at 400 Hz on a Bruker Avance DRX-400 FT spectrometer in DMSO-d_6. Chemical shifts are reported in ppm downfield from TMS as internal standard; coupling constants *J* are given in Hz. The mass spectra were recorded on a Varian Mat, CH-7 at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyser.

All chemicals were purchased from Sigma, Fluka or Merck Co. *S. aureus* ATCC 1112 was purchased from the Pasteur Institute of Iran and *S. aureus* (methicillin-resistant) was isolated from different specimens which were referred to the Microbiological Laboratory, Ghaem Hospital, Medical University of Mashhad-Iran. Its methicillin resistance was tested according to the NCCLS guidelines.[5]



4a: R= 4-$OMeC_6H_4$ **4b**: R= 4-MeC_6H_4 **4c**: R= 4-ClC_6H_4 **4d**: R= 4-BrC_6H_4 **4e**: R= 3-ClC_6H_4 **4f**: R= 4-$NO_2C_6H_4$ **4g**: R= C_6H_5 **4h**: R= CH_3

Scheme 1

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Table 1 Antibacterial activity (MIC, μg mL^{-1}) of 5-[(X-benzoylamino)]-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolecarboxamides **4a–h** against MRSA and MSSA

Compd	MRSA	MSSA	Compd	MRSA	MSSA
1 and 2	–	–	**4f**	4.0	4.0
4a	10.0	10.0	**4g**	75.3	75.9
4b	42.0	42.0	**4h**	99.4	99.4
4c	39.0	35.3	Erythromycin	32.0	32.0
4d	29.4	29.4	Cloxacillin	94.0	13.7
4e	45.3	45.3	Cephalexin	72.0	4.6

MRSA, methicillin-resistant *Staphylococcus aureus,*
MSSA, methicillin-susceptible *Staphylococcus aureus.*

Synthesis of 5-[(x-benzoylamino)]-1-(2,4-dinitrophenyl)-1H-4-pyrazolecarboxamides **4a–h***; general procedure*

A mixture of ethoxymethylenmalononitrile (12.2 g, 100 mmol) and 2,4-dinitrophenylhydrazine (19.8 g, 100 mmol) in ethanol (200 mL) was heated under reflux for 4 h. After cooling, the crystals that formed were filtered off, washed with ethanol and dried at 70 °C to give compound **1** (18.4 g, 67%, m.p. 217–220 °C), [lit.[3] 218–220 °C].

Compound **1** (34 mmol) was gradually added during 1 h, with stirring to concentrated sulfuric acid (30 mL), which was kept in an ice-bath. The inside temperature was kept between 15 and 20 °C. The solution was stirred at rt for a further 4 h, then water (40 mL) was added to the solution in an ice-bath and it was stirred for a further 2 h before it was poured onto crushed ice; finally it was neutralised with dilute aqueous NaOH. The reaction mixture which was allowed to reach 50–70 °C during the neutralisation, was cooled to rt, filtered and washed with water and then CH_2Cl_2, to give the pure product **2** (9.2 g, 93%, m.p. 246–247 °C), [lit.[4] 246–248 °C].

Acid chloride (**3a–h**) (12 mmol) was added dropwise at room temperature to a stirred solution of **2** (2.74 g, 10 mmol) in dry pyridine (20 mL). 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 crystallised to provide the pure desired compound **4a–h**.

1-(2,4-Dinitrophenyl)-5-[(4-methoxybenzoyl)amino]-1H-4-pyrazolecarboxamide (**4a**): Light yellow crystals (ethanol). Yield, 69%; m.p. 275–277 °C; IR: 1667, 1690 (C=O), 3243 (NH) and 3183, 3395 (NH_2) cm^{-1}; ^{1}H NMR: δ = 3.86 (s, 3H), 6.97 (d, 2H, *J* = 8.5), 7.77 (d, 2H, *J* = 8.5), 8.13 (d, 1H, *J* = 8.8 Hz), 8.17 (s, 1H), 8.45 (br s, 1H), 8.60 (dd, 1H, *J* = 8.8 Hz, *J′* = 2.4 Hz), 8.73 (br s, 1H), 8.88 (br s, 1H), 8.95 (d, 1H, *J* = 2.4 Hz); MS (70 eV): *m/z* = 426 (M^+). Anal. Calcd for $C_{18}H_{14}N_6O_7$ (426.34): C, 50.71; H, 3.31; N, 19.71. Found: C, 50.59; H, 3.25; N, 20.01%.

1-(2,4-Dinitrophenyl)-5-[(4-methylbenzoyl)amino]-1H-4-pyrazolecarboxamide (**4b**): Light yellow crystals (ethanol). Yield, 73%; m.p. 260–262 °C; IR: 1667, 1690 (C=O), 3228 (NH) and 3185, 3390 (NH_2) cm^{-1}; ^{1}H NMR: δ = 2.43 (s, 3H), 7.43 (d, 2H, *J* = 8.3), 7.75 (d, 2H, *J* = 8.3), 8.27 (d, 1H, *J* = 8.8 Hz), 8.31 (s, 1H), 8.55 (dd, 1H, *J* = 8.8 Hz, *J′* = 2.4 Hz), 8.77 (br s, 1H), 8.95 (br s, 1H), 8.99 (d, 1H, *J* = 2.4 Hz), 9.45 (br s, 1H); MS (70 eV): *m/z* = 410 (M^+). Anal. Calcd for $C_{18}H_{14}N_6O_6$ (410.34): C, 52.69; H, 3.44; N, 20.48. Found: C, 52.49; H, 3.37; N, 20.65%.

5-[(4-Chlorobenzoyl)amino]-1-(2,4-dinitrophenyl)-1H-4-pyrazolecarboxamide (**4c**): Light yellow crystals (ethanol). Yield, 65%; m.p. 284–285 °C; IR: 1690, 1704 (C=O), 3217 (NH) and 3185, 3390 (NH_2) cm^{-1}; ^{1}H NMR: δ = 7.48 (d, 2H, *J* = 8.4), 7.73 (d, 2H, *J* = 8.4), 8.07 (d, 1H, *J* = 8.8 Hz), 8.23 (s, 1H), 8.67 (dd, 1H, *J* = 8.8 Hz, *J′* = 2.4 Hz), 8.73 (br s, 1H), 8.85 (br s, 1H), 8.91 (d, 1H, *J* = 2.4 Hz), 9.12 (br s, 1H); MS (70 eV): *m/z* = 432 (M^++2). Anal. Calcd for $C_{17}H_{11}N_6O_6Cl$ (430.76): C, 47.40; H, 2.57; N, 19.51. Found: C, 47.23; H, 2.49; N, 19.65%.

5-[(4-Bromobenzoyl)amino]-1-(2,4-dinitrophenyl)-1H-4-pyrazolecarboxamide (**4d**): Light yellow crystals (ethanol). Yield, 71%; m.p. 299–301 °C; IR: 1690, 1703 (C=O), 3217 (NH) and 3185, 3390 (NH_2) cm^{-1}; ^{1}H NMR: δ = 7.77 (d, 2H, *J* = 8.3), 7.87 (d, 2H, *J* = 8.3), 8.17 (d, 1H, *J* = 8.8 Hz), 8.33 (s, 1H), 8.77 (dd, 1H, *J* = 8.8 Hz, *J′* = 2.4 Hz), 8.85 (br s, 1H), 8.92 (d, 1H, *J* = 2.4 Hz), 8.97 (br s, 1H), 10.50 (br s, 1H); MS (70 eV): *m/z* = 477 (M^++2). Anal. Calcd for $C_{17}H_{11}N_6O_6Br$ (475.21): C, 42.97; H, 2.33; N, 17.68. Found: C, 42.73; H, 2.23; N, 17.91%.

5-[(3-Chlorobenzoyl)amino]-1-(2,4-dinitrophenyl)-1H-4-pyrazolecarboxamide (**4e**): Light yellow crystals (ethanol). Yield, 55%; m.p. 251–253 °C; IR: 1690, 1705 (C=O), 3221 (NH) and 3185, 3390 (NH_2) cm^{-1}; ^{1}H NMR: δ = 7.35–7.39 (m, 1H), 7.54 (1H, t, *J* = 7.9 Hz), 7.74–7.80 (m, 2H), 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), 8.87 (br s, 1H), 8.99 (br s, 1H), 9.30 (br s, 1H); MS (70 eV): *m/z* = 432 (M^++2). Anal. Calcd for $C_{17}H_{11}N_6O_6Cl$ (430.76): 47.40; H, 2.57; N, 19.51. Found: C, 47.21; H, 2.50; N, 19.31%.

1-(2,4-Dinitrophenyl)-5-[(4-nitrobenzoyl)amino]-1H-4-pyrazolecarboxamide (**4f**): Yellow crystals (ethanol). Yield, 60%; m.p. 306–307 °C; IR: 1690, 1706 (C=O), 3220 (NH) and 3185, 3390 (NH_2) cm^{-1}; ^{1}H NMR: δ = 7.69 (d, 2H, *J* = 8.0 Hz), 7.78 (d, 2H, *J* = 8.0 Hz), 8.22 (d, 1H, *J* = 8.8 Hz), 8.29 (s,1H), 8.75 (dd, 1H, *J* = 8.8 Hz, *J´* = 2.4 Hz), 8.85 (d, 1H, *J* = 2.4 Hz), 8.89 (br s, 1H), 9.05 (br s, 1H), 10.29 (br s, 1H); MS (70 eV): *m/z* = 441 (M^+). Anal. Calcd for $C_{17}H_{11}N_7O_8$ (441.31): C, 46.27; H, 2.51; N, 22.22. Found: C, 46.03; H, 2.43; N, 22.34%.

5-(Benzoylamino)-1-(2,4-dinitrophenyl)-1H-4-pyrazolecarboxamide (**4g**): Light yellow crystals (ethanol). Yield, 70%; m.p. 239–240 °C; IR: 1690, 1704(C=O), 3225 (NH) and 3185, 3390 (NH_2) cm^{-1}; ^{1}H NMR: δ = 7.35–7.65 (m, 5H), 8.05 (d, 1H, *J* = 8.8 Hz), 8.11 (s, 1H), 8.28 (br s, 1H), 8.65 (dd, 1H, *J* = 8.8 Hz, *J´* = 2.4 Hz), 8.88 (d, 1H, *J* = 2.4 Hz), 8.97 (br s, 1H), 9.09 (br s, 1H); MS (70 eV): *m/z* = 396 (M^+). Anal. Calcd for $C_{17}H_{12}N_6O_6$ (396.32): C, 51.52; H, 3.05; N, 21.21. Found: C, 51.35; H, 2.95; N, 21.01%.

5-(Acetylamino)-1-(2,4-dinitrophenyl)-1H-4-pyrazolecarboxamide (**4h**): Light yellow crystal (ethanol). Yield, 77%; m.p. 320–321 °C; IR: 1690, 1695(C=O), 3221 (NH) and 3185, 3390 (NH_2) cm^{-1}; ^{1}H NMR: δ = 2.01 (s, 3H), 8.05 (d, 1H, *J* = 8.8 Hz), 8.35 (s, 1H), 8.46 (dd, 1H, *J* = 8.8 Hz, *J´* = 2.4 Hz), 8.73 (d, 1H, *J* = 2.4 Hz), 8.87 (br s, 1H), 8.94 (br s, 1H), 10.67 (br s, 1H); MS (70 eV): *m/z* = 334 (M^+). Anal. Calcd for $C_{12}H_{10}N_6O_6$ (334.25): C, 43.12; H, 3.02; N, 25.14. Found: C, 42.90; H, 2.93; N, 25.40%.

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References

1 M. Rahimizadeh, M. Pordel, M. Bakavoli, Sh. Rezaeian and A. Sadeghian, *World. J. Microbiol. Biotechnol*., 2010, **26**, 317.
2 H. Sadeghian, A. Sadeghian, M. Pordel, M. Rahimizadeh, P. Jahandari, A. Orafaie and M. Bakavoli, *Med. Chem. Res*., 2010, **19**, 103.
3 A. Kreutzberger and K. Burgwitz, *J. Heterocycl. Chem*., 1980, **17**, 265.
4 M. Pordel, Synthesis of new derivatives of aminopyrazolocarbonitrile, 2004, MSc thesis, Ferdowsi, University of Mashhad, Mashhad, Iran.
5 S.M. Finegold and L. Garrod, *Bailey and Scott's diagnostic microbiology*, C.V. Mosby Co., Toronto, 1995, 3rd edn., Chap. 13, pp. 171–193.

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