European Journal of Medicinal Chemistry 45 (2010) 647–650

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Molecular iodine promoted synthesis of new pyrazolo[3,4-d]pyrimidine derivatives as potential antibacterial agents

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ARTICLE INFO

Article history:
Received 8 April 2009
Received in revised form
21 October 2009
Accepted 30 October 2009
Available online 6 November 2009

Keywords:
Pyrazolo[3,4-d]pyrimidine
Antibacterial agents
Iodine
Heterocyclization

ABSTRACT

Iodocyclization of 5-amino-1-(2,4-dinitrophenyl)-1H-4-pyrazolcarboxamides with aromatic aldehydes gave a new series of pyrazolo[3,4-d]pyrimidine derivatives in a single step and their antibacterial activity comparable to Streptomycin as reference drug was evaluated.

1. Introduction

Fused pyrimidinone derivatives have attracted the attention of numerous researchers over many years due to their important biological activities. The structural similarity of pyrazolo[3,4-d]pyrimidines with purines [1] have made them a prime target for scientific research and in this context several reports dealing with the synthesis of these fused heterocyclic compounds have appeared in the literature [1–5,7–15]. An array of biological activities such as antibacterial, antifungal [2,3], antiphlogistic, antitumor [4] and herbicidal [5] has been reported to be shown by various pyrazolopyrimidines. It has been proved that these heterocyclic compounds are effective as inhibitors of inflammatory mediators in intact cells [6], anti-M. tuberculosis [7] and human enterovirus [8]. They also show inhibitory activity towards both tubulin polymerization and cyclin-dependent kinase [9] and enzymatic assays on Src and Abl tyrosine kinases [10]. Prompted by these claims and in continuing our synthetic studies on bioactive heterocycles [16], we have now synthesized a new series of pyrazolo[3,4-d]pyrimidine derivatives via molecular iodine catalyzed oxidative cyclization of 5-amino-1-(2,4-dinitrophenyl)-1H-4-pyrazolcarboxamides with aromatic aldehydes in a single step with the aim of evaluating their biological activities.

2. Results and discussion

2.1. Chemistry

The starting 5-amino-1-(2,4-dinitrophenyl)-1H-4-pyrazolcarbo nitrile (**1**) was obtained according to the previously published method [17]. Concentrated sulfuric acid mediated hydrolysis of this compound gave the corresponding 5-amino-1-(2,4-dinitrophenyl)-1H-4-pyrazolcarboxamide (**2**). Oxidative cyclization of the latter compound (**2**) with various substituted aromatic aldehydes in the presence of equimolar molecular iodine as a mild Lewis acid and oxidant [18] under neutral conditions in boiling acetonitrile gave a new series of pyrazolo[3,4-d]pyrimidine derivatives (**3a**–**j**) in good to excellent yields. (Scheme 1).

The structures of these compounds were confirmed from their spectral and micro analytical data. For example, the ^{1}H NMR spectrum of compound (**3c**) did not show the signal at δ 6.8 ppm belonging to NH_2 moiety of the precursor but instead showed a singlet for NH proton at δ 12.4 ppm which was removed on deuteration. Furthermore, the spectrum showed an AB quartet peak at the aromatic region, confirming the presence of an aromatic ring and the occurrence of heterocyclization. The IR spectrum was

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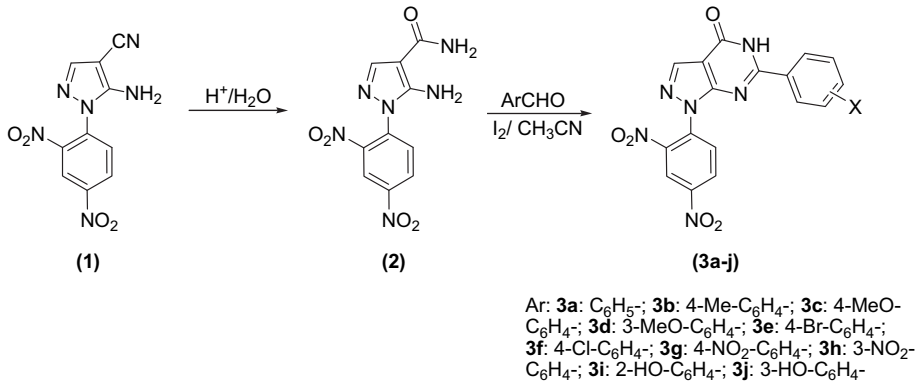
doi:10.1016/j.ejmech.2009.10.051



Scheme 1.

also devoid of the stretching vibration bands resembling the NH_2 moiety of the precursor but instead exhibited only one band at 3320 cm^{-1} for the NH moiety. The molecular ion peak of compound (**3c**) was observed at 408 (M^+) corresponding to the molecular formula $C_{18}H_{12}N_6O_6$.

2.2. Biological activities

The in vitro antibacterial activity of the newly synthesized compounds (**3a–j**) were screened for the antibacterial activity against several pathogenic representative Gram-positive bacteria (*Staphylococcus aureus* PTCC 1074 and *Bacillus subtilis* PTCC 1365); Gram-negative bacteria (*Escherichia coli* HB101 BA 7601C and *Pseudomonas aeruginosa* PTCC 1431) using disc diffusion sensitivity test [19,20]. Mueller–Hinton agar media were sterilized (15 min at 121 °C) and poured into the plates to a uniform depth of 5 mm and allow it to solidify. The microbial suspension (1.2×10^8 CFU/mL) (0.5 McFarland Nephelometery Standards) was streaked over the surface of media using a sterile cotton swab (15 min at 180 °C) to ensure confluent growth of the organisms. The tested compounds were dissolved in DMF and diluted with ethanol to get a solution of 100–600 $\mu g\,mL^{-1}$ concentration. The discs measuring 6.25 mm in diameter (Whatman no. 1 filter paper) were impregnated with prepared solution of compounds (**3a–j**) by 1 mL of the chemical solution which was added to each bottle contained 12 discs and placed on Muller-Hinton agar media previously inoculated with bacterial suspension. The inhibition zones as a criterion for anti-microbial activity were measured in millimeter at the end of an incubation period of 24 h at 37 °C. The results of these evaluations are given in Table 1. Streptomycin (binds to the 16SrRNA of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit therefore prevents initiation of protein synthesis and leads to death of microbial cell) was chosen as a standard drug at a concentration of 10 $\mu g\,ml^{-1}$. Streptomycin is an antibiotic that inhibits both gram-positive and gram-negative bacteria, and is therefore a useful broad spectrum antibiotic.

As it can be concluded from the data in Table 1, the compounds bearing 4-Br–C_6H_4– (**3e**) and 2-HO–C_6H_6– (**3i**) substituents has shown the highest sensitivity against *E. coli* and *P. aeruginosa*, respectively. Compounds (**3h**) and (**3j**) with other substituents of 3-NO_2–C_6H_4– and 3-HO–C_6H_4– respectively exhibited the best activity against the *S. aureus* strains while *B. Subtilis* has been more sensitive against compound (**3f**) with substituent 4-Cl–C_6H_4–. Therefore the good activity can be attributed to the presence of groups 4-bromo, 2 and 3-hydroxy and 4-chloro which are directly attached to the phenyl ring of the diazine system. All the other compounds were found to exhibit slight to moderate activities against the mentioned organisms. These results clearly demonstrate that hydroxyl or halogen substituted derivatives exhibited better activity compared to other substituted pyrazolo[3,4-d]pyrimidines.

3. Experimental

Melting points were recorded on an Electrothermal type 9100 melting point apparatus and are not corrected. The ^1H NMR

Table 1
Antibacterial data of the synthesized compounds **3a–j.**

Compound	Gram-negative bacteria		Gram-positive bacteria	
	Escherichia coli HB101 BA 7601C	*Pseudomonas aeruginosa* PTCC 1431	*Staphylococcus aureus* PTCC 1074	*Bacillus subtilis* PTCC 1365
3a	15[a] (+)[b] (400)[c]	9.5 (−) (400)	9 (−) (400)	10 (+) (400)
3b	9 (−) (200)	7.5 (−) (200)	13 (+) (200)	9 (−) (200)
3c	9.5 (−) (100)	12.5 (+) (100)	10 (−) (100)	10 (−) (100)
3d	11.5 (−) (100)	12.5 (+) (100)	9 (−) (100)	8 (−) (100)
3e	17 (++) (100)	10 (−) (100)	9 (−) (100)	9 (−) (100)
3f	10.5 (−) (400)	8 (−) (400)	8.5 (−) (400)	15 (++) (400)
3g	9 (−) (300)	13.5 (+) (300)	8.5 (−) (300)	9 (−) (300)
3h	8 (−) (400)	9 (−) (400)	15 (++) (400)	11 (−) (400)
3i	7 (−) (500)	16 (++) (500)	9 (−) (500)	9 (−) (500)
3j	12 (+) (500)	9.5 (−) (500)	16 (++) (500)	12 (+) (500)
Streptomycin (standard)	17	10	15	10

[a] Zones of inhibition in millimeter.
[b] (++) Highly sensitive; (+) Moderately sensitive; (−) Slightly sensitive.
[c] concentration in $\mu g\,mL^{-1}$ and the maximum inhibition zone for each compound has been shown. Disks of each concentration were placed in triplicate in nutrient agar medium seeded with fresh bacteria separately and the average was reported.

(100 MHz) spectra were recorded on a Bruker AC 100 spectrometer. Chemical shifts are reported in ppm downfield from TMS as internal standard; coupling constants *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.

Compound (**1**) was obtained according to the published method [21]. Other reagents were commercially available.

3.1. Synthesis of 5-amino-1-(2,4-dinitrophenyl)-1H-4-pyrazol carboxamide (**2**)

5-amino-1-(2,4-dinitrophenyl)-1H-4-pyrazole carbonitrile (**1**) was hydrolyzed according to lit. [21]. The resulting solid was recrystallized from ethanol-water. (Yield = 86%, m.p. = 234 °C, lit. [22] 234–235 °C).

3.2. General procedure for the synthesis of 6-aryl-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidine-4-one (**3a–j**)

To a mixture of 5-amino-1-(2,4-dinitrophenyl)-1H-4-pyrazol carboxamide (**2**) (0.2 mmol, 0.06 g) and various aromatic aldehydes (0.2 mmol) in dry acetonitrile (5 ml), molecular iodine (0.22 mmol, 0.057 g) was added. Then the mixture was refluxed and the progress of the reaction was monitored by TLC using chloroform-methanol (8:2) as eluent. After the reaction was completed, the mixture was cooled to room temperature. A solution of sodium-thiosulphate (5%) was added and the resulted solid was filtered off and washed with water. The crude product was recrystallized from ethanol.

3.2.1. 1-(2,4-Dinitrophenyl)-6-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3a**)

yield = 78%, m.p. = 260 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 7.52 (m, 5H), 7.73 (s, 1H), 8.35 (d, J = 8.5 Hz, 1H), 8.75 (d, J = 8.7 Hz, 1H), 8.95 (s, 1H), 12.6 (br s, 1H, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3320 (NH), 1650 (C = O); Mass spectrum, *m*/*z*: 378 (M^+); Anal. Calcd. for $C_{17}H_{10}N_6O_5$ (%): C, 53.97; H, 2.66; N, 22.22; Found: C, 53.55; H, 2.45; N, 22.01.

3.2.2. 1-(2,4-Dinitrophenyl)-6-(4-Methylphenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3b**)

yield = 70%, m.p. = 324–325 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 2.32 (s, 3H, CH_3), 7.22 (d, J = 8.0 Hz, 2H), 7.42 (d, J = 8.0 Hz, 2H), 7.74 (s, 1H), 8.35 (d, J = 8.4 Hz, 1H), 8.75 (d, J = 8.7 Hz, 1H), 8.95 (s, 1H), 12.6 (br s, 1H, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3330 (NH), 1660 (C═O); Mass spectrum, *m*/*z* 392 (M^+); Anal. Calcd. for $C_{18}H_{12}N_6O_5$ (%): C, 55.11; H, 3.08; N, 21.42; Found: C, 54.95; H, 2.90; N, 21.22.

3.2.3. 1-(2,4-Dinitrophenyl)-6-(4-Methoxyphenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3c**)

yield = 70%, m.p. = 328 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 3.84 (s, 3H, OCH_3), 6.94 (d, J = 9.0 Hz, 2H), 7.22 (d, J = 9.0 Hz, 2H), 7.73 (s, 1H), 8.35 (d, J = 8.5 Hz, 1H), 8.75 (d, J = 8.8 Hz, 1H), 8.95 (s, 1H), 12.4 (br s, 1H, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3320 (NH), 1650 (C═O); Mass spectrum, *m*/*z* 408 (M^+); Anal. Calcd. for $C_{18}H_{12}N_6O_6$ (%): C, 52.95; H, 2.96; N, 20.58; Found: C, 52.70; H, 2.83; N, 20.29.

3.2.4. 1-(2,4-Dinitrophenyl)-6-(3-Methoxyphenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3d**)

yield = 73%, m.p. = 279 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 3.77 (s, 3H, OCH_3), 7.32 (4H, m), 7.75 (s, 1H), 8.35 (d, J = 8.4 Hz, 1H), 8.75 (d, J = 8.7 Hz, 1H), 8.95 (s, 1H), 12.7 (br s, 1H, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3300 (NH), 1650 (C═O); Mass spectrum, *m*/*z* 408 (M^+); Anal. Calcd. for $C_{18}H_{12}N_6O_6$ (%): C, 52.95; H, 2.96; N, 20.58; Found: C, 52.55; H, 2.77; N, 20.43.

3.2.5. 6-(4-Bromophenyl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3e**)

yield = 74%, m.p. = 298–300 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 7.74 (s, 1H), 7.70 (d, J = 7.2 Hz, 2H), 8.21 (d, J = 7.2 Hz, 2H), 8.35 (d, J = 8.5 Hz, 1H), 8.75 (d, J = 8.8 Hz, 1H), 8.95 (s, 1H), 12.9 (br s, 1H, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3330 (NH), 1680 (C═O); Mass spectrum, *m*/*z* 457 (M^+); Anal. Calcd. for $C_{17}H_9BrN_6O_5$ (%): C, 44.66; H, 1.98; N, 18.38; Found: C, 44.23; H, 1.88; N, 18.03.

3.2.6. 6-(4-Chlorophenyl)-1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3f**)

yield = 87%, m.p. = 346–347 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 7.73 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 8.15 (d, J = 8.4 Hz, 2H), 8.35 (d, J = 8.4 Hz, 1H), 8.75 (d, J = 8.7 Hz, 1H), 8.95 (s, 1H), 12.7 (br s, 1H, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3320 (NH), 1680 (C═O); Mass spectrum, *m*/*z* 412 (M^+); Anal. Calcd. for $C_{17}H_9ClN_6O_5$ (%): C, 49.47; H, 2.20; N, 20.36; Found: C, 49.34; H, 2.13; N, 20.12.

3.2.7. 1-(2,4-Dinitrophenyl)-6-(4-nitrophenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3g**)

yield = 88%, m.p. = 350 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 7.76 (s, 1H), 7.89 (d, J = 9.1 Hz, 2H), 8.35 (d, J = 9.1 Hz, 2H), 8.35 (d, J = 8.5 Hz, 1H), 8.75 (d, J = 8.9 Hz, 1H), 8.95 (s, 1H), 13.3 (br s, 1H, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3310 (NH), 1670 (C═O); Mass spectrum, *m*/*z* 423 (M^+); Anal. Calcd. for $C_{17}H_9N_7O_7$ (%): C, 48.24; H, 2.14; N, 23.16; Found: C, 47.98; H, 2.01; N, 22.90.

3.2.8. 1-(2,4-Dinitrophenyl)-6-(3-nitrophenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3h**)

yield = 81%, m.p. = 338–339 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 7.75 (s, 1H), 8.25 (4H, m), 8.35 (d, J = 8.5 Hz, 1H), 8.75 (d, J = 8.8 Hz, 1H), 8.95 (s, 1H), 13.3 (br s, 1H, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3320 (NH), 1660 (C═O); Mass spectrum, *m*/*z* 423 (M^+); Anal. Calcd. for $C_{17}H_9N_7O_7$ (%): C, 48.24; H, 2.14; N, 23.16; Found: C, 48.04; H, 2.11; N, 22.97.

3.2.9. 1-(2,4-Dinitrophenyl)-6-(2-Hydroxyphenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3i**)

yield = 69%, m.p. = 302 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 7.72 (s, 1H), 7.12 (4H, m), 8.35 (d, J = 8.3 Hz, 1H), 8.75 (d, J = 8.7, 1H), 8.95 (s, 1H), 9.54 (br s, 1H, OH, D_2O exchangeable), 13.3 (br s, 1H, NH, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3300 (NH), 1660 (C═O); Mass spectrum, *m*/*z* 394 (M^+); Anal. Calcd. for $C_{17}H_{10}N_6O_6$ (%): C, 51.78; H, 2.56; N, 21.31; Found: C, 51.58; H, 2.44; N, 21.14.

3.2.10. 1-(2,4-Dinitrophenyl)-6-(3-Hydroxyphenyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**3j**)

yield = 68%, m.p. = 316 °C, ^{1}H NMR spectrum in DMSO-d_6 (δ, ppm): 7.18 (4H, m), 7.73 (s, 1H), 8.35 (d, J = 8.4 Hz, 1H), 8.75 (d, J = 8.7 Hz, 1H), 8.95 (s, 1H), 9.89 (br s, 1H, D_2O exchangeable), 13.3 (br s, 1H, D_2O exchangeable); IR spectrum (ν_{max}, cm^{-1}): 3290 (NH), 1650 (C═O); Mass spectrum, *m*/*z* 394 (M^+); Anal. Calcd. for $C_{17}H_{10}N_6O_6$ (%): C, 51.78; H, 2.56; N, 21.31; Found: C, 51.66; H, 2.43; N, 21.25.

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