New pyrazolo[1,5-a]pyrimidine and pyrazolo[5,1-c]-1,2,4-triazine derivatives as potential antimicrobial agents

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ABSTRACT

A series of new pyrazolopyrimidines 4a,b, 5, 8, and 11 were prepared by reaction of 3,5-diaminopyrazole 2 with active methylene reagents, arylidenemalononitrile 6 or ethyl 2-cyano-3,3-bis(methylthio)acrylate 9. Also new pyrazolo[5,1-c]-[1,2,4]triazine 13 was prepared by reaction of 1-nitroso-2-naphthol 12 with 2. The antibacterial and antifungal activities were screened for the new compounds and compound 11 exhibited the highest potency against all tested organisms.

Key words: 3,5-diaminopyrazole, pyrazolopyrimidine, pyrazolotriazine, antimicrobial activity

INTRODUCTION

The reaction of binucleophiles of aminoazole-type with electrophiles are the most synthetic approach for obtaining diverse heterocyclic systems containing azole moiety [1,2]. The most investigated area of aminoazole chemistry is their two-component reactions with ketoesters, β-dicarbonyls, or α,β-unsaturated aldehydes and ketones yielding fused azoloazines. In addition, pyrazole derivatives have attracted continuing interest over the years because of their varied biological and pharmacological activities [3], and also find application in photography [4] and as dyes [5]. The aminopyrazole compounds have been easily obtained by the reaction of nitrile derivatives with hydrazine hydrate [6-8], and are very useful as precursors for the synthesis of fused heterocyclic ring systems. The present work is a complements our previous research articles to discover new antimicrobial agents [9,10].

EXPERIMENTAL SECTION

Chemistry
Melting points were measured on an electrothermal Gallenkamp melting point apparatus. Elemental analyses were carried out at the Microanalytical Unit, Cairo University, Giza, Egypt; the results were in satisfactory agreement with the calculated values. The IR spectra were recorded in KBr disks on a Mattson 5000 FTIR spectrometer (not all frequencies are reported). The NMR spectra were acquired using a Bruker WP 300 spectrometer at 300 MHz using TMS as an internal standard and CDCl₃ or DMSO-d₆ as solvent. The Mass spectra were performed using a Varian MAT 311 mass spectrometer at 70 eV. Antibacterial activities were carried out at the laboratory of the microbiology unit - Botany Department - Faculty of Science - Mansoura University, Egypt. 4-(4-acetyl-phenylazo)-3,5-diamino-1H-pyrazole 2 [11] and ethyl 2-cyano-3,3-bis(methylthio)acrylate 9 [12] were prepared according to literature.

Synthesis of compounds 4a and 4b
A mixture of 2 (0.005 mol) and acetyl acetone (3a) or ethyl acetoacetate (3b) (0.005 mol) in acetic acid (20 ml) and sodium acetate (0.60 g) was heated under reflux for 3-4 hours. The reaction mixture was cooled to room temperature and poured into ice-water. The separated solid was filtered, washed with hot ethanol, dried and recrystallized from DMF to give 4a or 4b.
3-(4-Acetyl-phenylazo)-2-amino-5,7-dimethyl-pyrazolo[1,5-a]-pyrimidine (4a):

3-(4-Acetyl-phenylazo)-2-amino-7-hydroxy-5-methyl-pyrazolo[1,5-a]-pyrimidine (4b):
Reddish brown crystals, m.p. = 199-200°C. Yield = 64%. IR (ν/cm⁻¹): 1658 (C=O), 3392, 3187 (NH₂), 3274 (OH). ¹H NMR (DMSO): δ/ppm = 2.25 (s, 3H, COCH₃), 2.45 (s, 3H, CH₃), 7.15 (s, 1H, pyrimidine C₃-H), 7.50 (d, 2H, Ar-H), 7.90 (d, 2H, Ar-H), 8.90 (s, 1H, OH). Anal.calcd. for C₁₅H₁₃N₅O₂ (310.31): C, 58.06; H, 4.55; N, 27.08. Found: C, 58.19; H, 4.48; N, 27.17.

3-(4-Acetyl-phenylazo)-2,5,7-triamino-pyrazolo[1,5-a]-pyrimidine (5):
A mixture of 2 (0.005 mol) in ethanol (20 ml), 5 drops of triethylamine and malononitrile (0.005 mol) was refluxed 4 hours. The reaction mixture was poured into ice water, and neutralized by HCl. The obtained solid was filtered off, dried and recrystallized from EtOH:DMF mixture (1:1) to give 5 as red-brown solids.

Brown crystals, m.p. > 300 °C. Yield = 68%. IR (ν/cm⁻¹): 1664 (C=O), 3408, 3363, 3205 (3NH₂). ¹H NMR (DMSO): δ/ppm = 2.30 (s, 3H, COCH₃), 7.10 (s, 1H, pyrimidine C₃-H), 7.25 (s, 2H, NH₂), 7.50 (d, 2H, Ar-H), 7.80 (d, 2H, Ar-H), 9.25 (s, 2H, NH₂), 10.40 (s, 2H, NH₂). Anal.calcd. for C₁₅H₁₄N₄O₂ (310.31): C, 54.19; H, 4.55; N, 36.11. Found: C, 54.41; H, 4.46; N, 36.22.

3-(4-Acetyl-phenylazo)-2,7-diamino-6-cyano-4,5-dihydro-5-oxo-pyrazolo[1,5-a]-pyrimidine (8):
To a suspension of compounds 2 (0.002 mol) and the arylidene-malononitrile (0.002 mol) in absolute ethanol (20 ml), five drops of triethylamine and malononitrile (0.005 mol) was refluxed 12 hours. The reaction mixture was cooled at room temperature and the solid precipitate was filtered off, dried and recrystallized from EtOH:DMF mixture (1:1) to give 8.

Brown crystals, m.p. = 193-194°C. Yield = 54%. IR (ν/cm⁻¹): 1662 (C=O), 2210 (C≡N), 3436, 3323, 3273 (2NH₂). MS (M⁺; EI): m/z (%) = 439 (25). Anal.calcd. for C₂₃H₁₄N₄O₂ (439.47): C, 62.86; H, 4.82; N, 28.68. Found: C, 62.64; H, 4.73; N, 28.54.

3-(4-Acetyl-phenylazo)-2-amino-6-cyano-5-oxo-pyrazolo[1,5-a]-pyrimidine (11):
A suspension of 2 (0.005 mol) in 30 ml dioxane was refluxed with ethyl 2-cyano-3,3-bis(methylthio)acrylate (9) (0.005 mol) and 0.5 ml triethylamine for 4 hours. The mixture was left to cool at room temperature. The crystals separating on cooling were filtered off and crystallized from EtOH:DMF mixture (1:1) to give 11.

Dark red crystals, m.p. = 274-275°C. Yield = 64%. IR (ν/cm⁻¹): 1671 (broad, C=C), 2201 (C≡N), 3436, 3323, 3241 (NH and NH₂). MS (M⁺; EI): m/z (%) = 367 (55). Anal.calcd. for C₁₆H₁₅N₅O₂S (367.39): C, 52.31; H, 3.57; N, 26.69. Found: C, 52.13; H, 3.64; N, 26.76.

2-Amino-1-(4-acetyl-phenylazo)-naptho[2,1-e]-pyrazolo[5,1-c]-[1,2,4]triazine (13):
A mixture of compound 2 (0.002 mol) and 1-nitroso-2-naphthol 12 (0.002 mol) in absolute ethanol (20 ml) refluxed for 12 hours. The reaction mixture was cooled at room temperature and the solid precipitate was filtered off, dried and recrystallized from the EtOH-DMF mixture (1:2) to give 13.

Brown powder, m.p. = 252-253°C. Yield = 54%. IR (ν/cm⁻¹): 1660 (C=O), 3393, 3263 (NH₂). ¹H NMR (DMSO): δ/ppm = 2.30 (s, 3H, COCH₃), 7.20-7.80 (m, 10H, Ar-H), 10.35 (s, 2H, NH₂). Anal.calcd. for C₂₁H₁₅N₆O₂ (381.39): C, 66.13; H, 3.96; N, 25.71. Found: C, 66.22; H, 3.88; N, 25.82

Antimicrobial activity
Chemical compounds were individually tested against a panel of gram positive and gram negative bacterial pathogens, yeast and fungi. Antimicrobial tests were carried out by the agar well diffusion method [13] using 100 µL of suspension containing 1x10⁶ CFU/mL of pathological tested bacteria and 1 x10⁶ CFU/ml of yeast spread on nutrient agar (NA) and Sabourand dextrose agar (SDA) respectively. After the media had cooled and solidified, wells (10 mm in diameter) were made in the solidified agar and loaded with 100 µL of tested compound solution prepared by dissolving 100 mg of the chemical compound in one ml of dimethyl sulfoxide (DMSO). The inoculated plates were then incubated for 24 h at 37 °C for bacteria and 48h at 28°C for fungi. Negative controls were prepared using DMSO employed for dissolving the tested compound. Ciprofloxacin (50 µg/ml) and Ketoconazole (50 µg/ml) were used as standard for antibacterial and antifungal activity respectively. After incubation time, antimicrobial
activity was evaluated by measuring the zone of inhibition against the test organisms and compared with that of the standard. The observed zone of inhibition is presented in Table 1. Antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated.

RESULTS AND DISCUSSION

Chemistry
The starting material 3,5-diaminopyrazole 2 was prepared according to the reported method [11], starting from 4-aminoacetophenone by diazotiazation followed by coupling with malononitrile to give the intermediate hydrazone 1. Refluxing of compound 1 with hydrazine hydrate in ethanol furnished the target precursor 2 (Scheme 1).

\[ \text{H}_2\text{C} \text{CN} + \text{NH}_2 \xrightarrow{(i) \text{NaNO}_2/\text{HCl}} \text{Me-C} \xrightarrow{(ii) \text{EtOH/NaOAc}} \text{N-N=CN} \]

Scheme (1)

The study was extended to investigate the behavior of 2 with active methylene reagents such as acetylacetonate, malononitrile and ethyl acetooacetate. Thus, when acetyl acetone (3a, R_1 = Me) or ethyl acetooacetate (3b, R_1 = OEt) was reacted with compound 2 in refluxing acetic acid containing sodium acetate, the reaction furnished the corresponding 2-amino-5,7-dimethyl-pyrazolopyrimidine 4a or 2-amino-3-arylazo-7-hydroxy-5-methyl pyrazolo[1,5-a]pyrimidine derivative 4b respectively (Scheme 2). The structures of compounds 4a and 4b were assigned on the basis of its elemental analyses and spectral data. The IR spectrum of 4a exhibited absorption bands for the carbonyl group (acetyl) at 1662 cm\(^{-1}\) and for the amino group at 3325 and 3266 cm\(^{-1}\). Its Mass spectrum showed the molecular ion peak at m/z = 308 (65%) corresponding to the molecular weight of the molecular formula C\(_{16}\)H\(_{16}\)N\(_6\)O.

The \(^1\)H NMR spectrum of 4b showed two singlet signals at 2.25 and 2.45 ppm corresponding to the protons of two methyl groups (COCH\(_3\) and pyrimidine-CH\(_3\)), singlet signal at 7.15 ppm for one proton (pyrimidine C\(_5\)-H), two doublet signals at 7.50 and 7.90 ppm for four aromatic protons, singlet signal at 8.90 ppm for the two amino protons (NH\(_2\)) and singlet signal at 9.40 ppm for the proton of the hydroxyl group.

Similarly, when compounds 2 were treated with malononitrile in refluxing ethanol containing drops of triethylamine, the reaction yielded the corresponding 2,5,7-triamino-pyrazolopyrimidine 5 (Scheme 2). The IR spectrum of 5 exhibited absorption band at 1664 cm\(^{-1}\) indicating the presence of carbonyl group (C=O), band at 2210 cm\(^{-1}\) due to CN group and absorption bands at 3407, 3354, 3273 cm\(^{-1}\) due to two amino groups (2NH\(_2\)).
Ethyl 2-cyano-3,3-bis(methylthio)acrylate 9 was reacted with 3,5-diaminopyrazole 2 under reflux in dioxane containing catalytic amounts of triethylamine to afford the corresponding pyrazolo[1,5-a]-pyrimidine 11. The formation of 11 from the reaction of 2 with the ketene dithioacetal 9 proceeds via initial alkylation of the ring nitrogen in 2 to give 10 followed by intramolecular cyclization to yield the final product 11 (Scheme 2). The IR spectrum of 11 revealed characteristic bands for C=O, C≡N and (NH & NH₂) functional groups at 1671, 2201 and (3436, 3323, 3241 cm⁻¹).
As an extension of our study, the behavior of 2 towards nitrosonaphthols as a convenient synthetic route to pyrazolo[5,1-c]-[1,2,4]triazine derivatives was also investigated. Treatment of 2 with 1-nitroso-2-naphthol in boiling ethanolic solution afforded a single product identified as 2-amino-3-arylazo-naphtho[2,1-e]pyrazolo[5,1-c]-[1,2,4]-triazine derivative 13 (Scheme 3).

Antimicrobial activity
The synthesized compounds 2, 4a, 4b, 5, 8, 11 and 13 were screened for their antibacterial and antifungal activities at 100 µg/mL concentration against two Gram positive bacteria (Staphelococcus Aureus ATCC 29213; B. subtilis ATCC6633), two Gram negative bacteria (Pseudomonas Aeroginosa ATCC 27953; E. coli ATCC 25922), Yeast (Candida albicans IMRU3669) and Filamentous Fungus (Aspergillus Niger ATCC 16404). Ciprofloxacin and Ketoconazole were respectively used as standard antibacterial and antifungal reference, respectively. Most of the tested compounds showed accepted antimicrobial activities with respect to the control drugs. The results of antimicrobial activities were shown in Table 1. Compound 11 exhibited the highest potency against all tested organisms with respect to reference drugs. Compound 11 inhibited the growth of S. Aureus ATCC 29213, P. Aeruginosa ATCC 27953 and E. coli ATCC 25922 with inhibition zones 23, 24 and 22 mm respectively.

Table 1: Antimicrobial activity expressed as inhibition diameter zones in millimeters (mm)

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
<th>Yeast</th>
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<tr>
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<td>S. Aureus</td>
<td>B. Subtilis</td>
<td>P. Aeruginosa</td>
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<td>Ketoconazole</td>
<td>N.A.</td>
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The experiment was carried out in triplicate and the average zone of inhibition was calculated; (N. A. = no activity).

CONCLUSION

Novel pyrazolo[1,5-a]pyrimidines and pyrazolo[5,1-c]-[1,2,4]triazines were prepared starting from 3,5-diaminopyrazole derivatives. The antimicrobial activity was screened for the new compounds.

REFERENCES