Synthesis of bioactive heterocycles

SYNTHESIS OF BIOACTIVE HETEROCYCLES FROM 6-AMINO-4-(2-CHLORO-5-NITROPHENYL)-3-METHYL-1,4-DIHYDROPYRANO[2,3-c]PYRAZOLE-5-CARBONITRILE


Keywords: Enaminonitrile, pyranopyrazole, pyrimidinone, oxazinone, antimicrobial agents.

Enaminonitrile derivative, 6-amino-4-(2-chloro-5-nitrophenyl)-3-methyl-1,4-di hydropyrano[2,3-c]pyrazole-5-carbonitrile (I) was synthesized. This compound was utilized as a building block for the synthesis of new 3-methylpyrazolopyran moiety incorporated with different heterocycles involving pyrimidinone, oxazinone, and iminopyrimidine, in addition to novel derivatives including diacetyl derivative (S), benzoyl derivative (6), carbamothioic acid (10) and urea derivative (13). Spectral techniques, FT-IR, 1H-NMR and mass spectroscopy and elemental analysis were used to characterize the synthesized compounds. Screening and evaluation of these products as antimicrobial agents showed that the derivatives 5, 6, 10, and 13 possess a potent activity.

Results and Discussion

Syntheses

The previously reported pyranopyrazole derivative (I) was allowed to react with different reagents aiming to synthesize antimicrobial heterocycles. Reaction of 1 with formic acid afforded the pyrimidinon e derivative 2 whose structure was confirmed from IR spectral data which revealed the absence of absorption bands of \(\text{C} = \text{N}\) and \(\text{NH}_2\) groups and the appearance of bands characteristic to carbonyl and NH groups at \(\nu_{1682} \text{ cm}^{-1}\) and \(3182 \text{ cm}^{-1}\), respectively. The \(1^H\)-NMR spectrum showed a singlet at \(\delta_{11.08} \text{ ppm}\), disappeared by \(\text{D}_2\text{O}\) due to \(\text{NH}\) group proton. Acid hydrolysis of the cyano functionality was carried out by addition of concentrated sulphuric acid onto pyranopyrazole derivative 1 at room temperature to give the amide derivative 3. The structure of the amide 3 was elucidated by the FTIR spectra which showed no absorption band of \(\text{C} = \text{N}\) and appearance of a new band due to \(\text{C}=\text{O}\) group at \(1685 \text{ cm}^{-1}\).

In our previously reported work for the synthesis of oxazinone derivatives,[6] the pyranopyrazole derivative I was allowed to react with acetic anhydride and/or benzoyl chloride under solvent-free conditions and afforded the pyrazolopyranooxazinones 4a, b. On the contrary, herein, the reaction of 1 with acetic anhydride in pyridine gave the diacetyl derivative 5 and benzoylation with benzoyl chloride in dry toluene as a solvent afforded the benzoyl derivative 6. The IR spectra of both products 5 and 6 revealed the presence of cyano group absorption that proved no cyclization has occurred (Scheme 1).

To make use of nucleophilic character of the amino group, it was subjected to react with various electrophiles. Thus, when enaminonitrile I was treated with triethyl orthoformate, it gave the imidoformate derivative 7. The latter product was utilized as a precursor for the synthesis of pyrazolopyranopyrimidine 8 by reaction with hydrazine hydrate in ethanol. The structure of 7 was confirmed from its IR spectrum that did not show the absorption frequency of \(\text{NH}_2\) group but showed the \(\text{C}=\text{O}\) group band at 1632 cm\(^{-1}\).
Scheme 1. Reactions of pyranopyrazole derivative.

Scheme 2. Further reactions of pyranopyrazole derivatives
Further, the $^{1}$H-NMR spectrum showed a singlet at $\delta$12.36 ppm which disappeared in D$_2$O and is due to NH group, a quartet peak owing to CH$_2$ group at $\delta$ 4.34-4.28 ppm and a triplet peak at $\delta$ 1.31-1.28 ppm due to CH$_3$ protons. The structure of 8 has been elucidated on the basis of IR spectrum which showed a coupling band at 3188, 3119 cm$^{-1}$ due to NH$_2$ group and two peaks for NH pyrazole and NH imino at 3349 and 3309 cm$^{-1}$, respectively. Its $^{1}$H-NMR spectrum showed a singlet at $\delta$ 12.57 ppm NH of pyrazole group and 10.25 ppm for C=NH. However, when the imidoformate derivative 7 was subjected to react with ammonium hydroxide in methanol, hydrolysis of the imidoformate functionality to the formamide derivative 9 occurred instead of the formation of the pyrimidinone derivative 2.

Further, treatment of the enaminitrile 1 with carbon disulfide afforded carbamothioic acid 10 instead of pyrimidenedithione derivative 11. The IR spectrum of 10 revealed the absorption band attributable to C=S group at 2215 cm$^{-1}$ and a sharp band at 1390 cm$^{-1}$ due to C=O group. The reaction of the compound 1 with phenylisocyanate in pyridine provided the urea derivative 13 instead of the pyrimidinone derivative 12. The structure of 13 was confirmed from its elemental and spectral analysis (Scheme 2).

**Antimicrobial Study**

The antibacterial activity of the synthesized compounds 2, 3, 7, 8, 10 and 13 was tested against a panel of two gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and two Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The antifungal activities of the compounds were tested against two fungi Candida albicans and Aspergillus flavus.

Each compound was dissolved in DMSO and a solution of concentration 1 mg mL$^{-1}$ were prepared. Separately paper discs (5cm) were cut and sterilized in an autoclave. The paper discs, soaked in the solution of the compound, were placed aseptically in the petri dishes containing nutrient agar media (agar 20g + beef extract 3g +peptone 5g) seeded with Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus flavus. The petri dishes were incubated at 36 °C and the inhibition zones were recorded after 24 h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic ampicillin and antifungal Colitrimazole was also recorded using the same procedure as above at the same concentration and solvents. At the end of the incubation period, the minimum inhibitory concentration (MIC) values were recorded as the lowest concentration of the substance that had no visible turbidity. Control experiments with DMSO and uninoculated media were run parallel to the test compounds under the same condition. The results of MIC measurements of the synthesized heterocycles compounds are shown in Table 2.

**Experimental**

All melting points were determined on an electrothermal apparatus and are uncorrected. The FT-IR were recorded in potassium bromide disks on Pye Unicam SP3-300 and Shimadzu FTIR 8101PC Infrared spectrophotometers. The $^{1}$H-NMR was recorded on a Varian Mercury VX-300 NMR spectrometer. $^{1}$H-NMR spectra were run at 300 MHz and on a Varian Gemini 200 MHz, Bruker AC 200 MHz using TMS as internal standard in deuterated chloroform (CDCl$_3$) or deuterated dimethyl sulfoxide (DMSO-d$_6$). Chemical shifts are quoted in $\delta$ and were related to that of the solvents. The mass spectra were recorded on a Shimadzu GC-MS QP1000 EX mass spectrometer at 70 eV. Elemental analyses were carried out at the Micro Analytical Center of Cairo University. All the reactions and the purity of the new compounds were followed and checked by TLC.

**Synthesis**

4-(2-Chloro-5-nitrophenyl)-3-methyl-4,6-dihydropyrazolo-[4',3',5:6]pyranono[2,3-d]pyrimidin-5(1H)-one (2)

A mixture of 1 (5 mmol, 1.66 g) and formic acid (20 mL) was refluxed for 2 h, the reaction mixture was poured after cooling into water and crushed ice, the solid formed was filtered off, washed with cold water and crystallized from ethanol to give compound 2 as a pale yellow solid (72 %), m.p. 229-230 °C. IR (KBr): 3403 (NH pyraz.), 3182 (NH pyrim.), 1682 (CO) cm$^{-1}$. MS m/z (%) 359 (M$^{+}$, 7.35), 360 (3.42), 230 (74.53), 179 (2.15), 43(100). $^{1}$H-NMR (DMSO-d$_6$) $\delta$ = 11.4 (s, 1H, NH), 11.08 (s, 1H, NH), 8.49 (s, 1H, CH, N = C2-H), 8.07-7.70 (m, 3H, aromatic), 4.64 (s, 1H, benzyl), 2.12 (s, 3H, CH$_3$). Anal. Calcd. for C$_{13}$H$_{16}$N$_3$O$_4$Cl (359.73): C, 20.08; H, 2.80; Cl, 9.85; N, 19.47. Found: C, 20.06; H, 2.82; Cl, 9.83; N, 19.48.

The MIC was determined using the disc diffusion technique by preparing discs containing 1.9-1000 µg/ml of each compound against gram positive Staphylococcus aureus and Bacillus subtilis and gram negative Escherichia coli and Pseudomonas aeruginosa. The antifungal activities of the compounds were tested against two fungi Candida albicans and Aspergillus flavus and applying the protocol. The two fold dilutions of the solution were prepared. The microorganism suspensions at 10 CF-U/mL (colony forming unit/ml) concentration were inoculated to the corresponding wells. The plates were incubated at 36°C for 24 h. for the bacteria. The standard antibiotic ampicillin and Antifungal Colitrimazole was also recorded using the same procedure as above at the same concentration and solvents.

Further, the $^{1}$H-NMR spectrum showed a singlet at $\delta$12.36 ppm which disappeared in D$_2$O and is due to NH group, a quartet peak owing to CH$_2$ group at $\delta$ 4.34-4.28 ppm and a triplet peak at $\delta$ 1.31-1.28 ppm due to CH$_3$ protons.

The antimicrobial activity of the synthesized heterocycles was shown in Table 1.

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**Minimum inhibitory concentration (MIC) measurements**

The % activity index for the compounds was shown in Table 2.
6-Amino-4-(2-chloro-5-nitrophenyl)-3-methyl-1,4-dihydropyrazino[2,3-c]pyrazole-5-carboxamide (3)

Compound 1 (5 mmol, 1.66 g) was added drop wise with stirring to concentrated cold sulphuric acid (6 mL) at 20 °C, the temperature did not exceed 40 °C during the addition, then the solution was stirred for further 1 h at room temperature and poured onto ice cold water (10 mL). The reaction mixture was left overnight in the refrigerator. The yellow precipitate was filtered off and crystallized from water to give compound 3 as a pale yellow solid (68 %), m.p. 175-176 °C. FTIR (KBr): 3588 (NH pyrazole), 3567-3370 (NH₂), 3191-3108(amide NH), 1685 (CO) cm⁻¹; MS m/z (%): 350(M⁺;1.36), 351(4.93), 307 (67.39), 230(70.44), 151 (43.39), 43(100). ¹H-NMR (DMSO-d₆) δ = 11 (s, 1H, NH, pyrazole, exchanged with D₂O), 7.38-6.67 (s, 4H, C₂-NH₂; CONH₂, exchanged with D₂O), 8.63-7.60 (m, 3H, aromatic), 4.68 (s, 1H, benzyl), 2.1 (s, 3H, CH₃). Anal. Calcd. for C₁₃H₁₁N₃OCl (350.72): C, 47.95; H, 3.16; Cl, 10.11; N, 15.98. Found: C, 47.93; H, 3.15; Cl, 10.11; N, 15.99.

N-Acetyl-N-[4-(2-chloro-5-nitrophenyl)-5-cyano-3-methyl-1,4-dihydropyrazino[2,3-c]pyrazol-6-yl]acetamide (5)

A solution of 1 (5 mmol, 1.66 g) in acetic anhydride-pyridine mixture (30 mL, 2.1 v/v) was heated on a water bath for 8 h, then cooled and poured into ice/ water mixture. The precipitate thus formed was filtered off, washed several times with water, dried and crystallized from dioxane to give compound 5, as a deep brown solid (50 %), m.p. > 300 °C. FTIR (KBr): 3355(NH pyrazole), 1787, 1739 (C=O), 2223(C=N) cm⁻¹. ¹H-NMR (DMSO-d₆) δ = 11.28 (s, 1H, NH, pyrazole, exchanged with D₂O), 8.42-7.70 (m, 3H, aromatic), 4.62 (s, 1H, benzyl), 2.12 (s, 6H, 2CH₃), 2.55 (s, 3H, CH₃). MS m/z (%): 415 (M⁺;100), 417(32), 416 (19.5). Anal. Calcd. for C₁₃H₁₁N₃OCl (415.79): C, 52; H, 3.39; Cl, 8.53; N, 16.84. Found: C, 52.01; H, 3.38; Cl, 8.52; N, 16.85.

Table 1. Antimicrobial study of the synthesized heterocycles compounds

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DIZ = Diameter of inhibition zone; % AI = % Activity index; NA = No activity

Table 2. Antimicrobial and antymycotic activities in terms of MIC (µg mL⁻¹)

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Ethyl-4-(2-chloro-5-nitrophenyl)-5-cyano-3-methyl-1,4-dihydropyrazino[2,3-c]pyrazol-6-yl]benzamidate (7)

A mixture of 1 (5 mmol, 1.66 g) and triethyl orthofoamte (20 mL) was refluxed for 24 h. After completion of the reaction, the excess of triethyl orthofoamte was removed under vacuum. The remaining solid was washed with n-hexane several times and crystallized from benzene to give
compound 7 was a pale brown solid (60 %), m.p. 233-234 °C. FTIR (KBr): 3180 (NH pyrazole), 1632 (C=NH), 2210 (C=N) cm⁻¹. ¹H-NMR (DMSO-d₆) δ = 12.36 (s, 1H, NH, pyrazole, exchanged with D₂O), 8.59 (s, 1H, N=CH), 8.17-7.77 (m, 3H, aromatic), 5.47 (s, 1H, benzyl), 4.34-4.28 (q, 2H, CH₂), 1.77 (s, 3H, CH₃, pyrazole), 1.31-1.28 (t, 3H, CH₃). MS m/z (%): 386.87M⁺ (11.08), 389.67 (5.07), 283(25.01), 259(34.62), 202(32.17), 146 (82.33), 82 (100). Anal. Calcld. for C₁₅H₁₀N₅O₆Cl (407.85): C, 44.17; H, 2.47; Cl, 6.89; N, 17.17. Found: C, 44.16; H, 2.46; Cl, 8.68; N, 17.18.

N-[4-(2-Chloro-5-nitrophenyl)-5-cyano-3-methyl-1,4-dihydropyrazino[2,3-d]pyrazol-6-yl]-N'-phenyl urea (13)

A mixture of 1 (10 mmol, 3.31 g) and phenylisocyanate (10 mmol) in pyridine (20 mL) was refluxed for 12 h. The reaction mixture was cooled and poured onto ice/ water mixture and neutralized with diluted HCl. The solid product so formed was collected by filtration and crystallized from methanol to give compound 13 as a deep yellow solid (56 %), m.p. > 300 °C. FTIR (KBr): 3371 (NH pyrazole), 3213, 3101 (2NH, amide), 1745 (C=O), 2210 (C=O) cm⁻¹. ¹H-NMR (DMSO-d₆) δ = 11.57 (s, 1H, NH, pyrazole), 8.67-7.11 (m, 2H, aromatic), 7.47 (s, 1H, benzyl), 8.9 (s, 1H, NH), 6.4 (s, 1H, CH), 1.9 (s, 3H, CH₃). MS m/z (%): 450 (M⁺, 3.47), 451.85 (490.), 244 (22.11), 219 (41.50), 198 (79.99). Anal. Calcld. for C₁₅H₁₀N₅O₆Cl (450.84): C, 55.95; H, 3.35; Cl, 7.86; N, 18.64. Found: C, 55.94; H, 3.33; Cl, 7.87; N, 18.65.

References


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