A Facile One-pot Synthesis of New Pyrazolopyrimidines and Pyrazolo Pyridines Derivatives
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ABSTRACT
A Simple Facile One-pot reaction, novel and efficient rout for the synthesis of substituted pyrazolo [3, 4-d] pyrimidines, and pyrazolo [3, 4-b] pyridines, results from reaction of substituted-5-amino-4-cyanopyrazoles with malononitrile and diethylmalonate respectively. The structures of the products and conceivable mechanisms are discussed. The antibacterial activity of some new synthesized compounds was evaluated and seemed to be significant.

Indexing terms/Keywords
substituted-5-amino-4-cyanopyrazoles; pyrazolo [3, 4-d] pyrimidines; pyrazolo [3, 4-b] pyridines; diethylmalonate; malononitrile and antibacterial activity.

Academic Discipline And Sub-Disciplines
Organic Chemistry

SUBJECT CLASSIFICATION
Organic Chemistry

TYPE (METHOD/APPROACH)
Some new nitrogen bridge-head pyrido[1,2-b][1,2,4]triazepines incorporating 6-methylchromone moiety have been synthesized from the reaction of 1,6-diamino-4-(6-methyl-4-oxo-4H-chromen-3-yl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (4) with some α,γ-bifunctional electrophiles including 2-cyano-3,3-bis(methylthio)acrylonitrile, 2-cyano-3,3-bis(methylthio)prop-2-enamide, 5-chloro-3-methyl-1-phenylpyrazole-4-carboxaldehyde, 2-chloro-3-formylquinoline, p-methoxybenzylidene-malononitrile, ethyl 2-cyano-3-(4-methoxyphenyl)prop-2-enate, chromone-3-carbonitrile. Structures of the newly synthesized products have been deduced upon the help of elemental analysis and spectral data. The synthesized compounds were screened for their antimicrobial activity.
INTRODUCTION

Pyrazolopyrimidines are of considerable chemical and pharmacological importance as purine analogues, it is a heterocyclic chemical compound with the molecular formula C_{m}H_{n}N_{p}. It forms the central core of a variety of more complex chemical compounds including some pharmaceuticals and pesticides. Pyrazolopyrimidines have antitumor, antileukemic activities. The pyrazole containing compounds have practical applications in the medicinal and agrochemical field and the biological activity of pyrazoles and its derivatives is well documented. The pyrazole ring has shown to be the basic moiety for a number of dyes, drugs and anesthetics. Amino and hydroxyl substituted pyrazoles have been used as choline esterase inhibitors. Pyrazolopyridines and its hydroderivatives are very interesting pyrazole derivatives with wide ranging biological activities. A number of pyrazole (3, 4-b) pyridines exhibit a wide range of biological activities, including interesting anxiolytic activity (e.g. trazolactone), dopamine D3 receptor antagonist, antherhetic and antiiallergic properties.

RESULTS AND DISCUSSION

The aim of this work, The 5-amino-4H-pyrazole-4-carbonitrile (1), were used as starting materials, which contains a cyano group in the ortho position is required for the synthesis of the condensed systems including pyridine and pyrimidine, with malononitrile and diethylmalonate.

In ethanolic sodium ethoxide solution, compounds (1a) and (1b) reacted with malononitrile to afford pale brown powders of mp 235-237 °C for (2a) and 289-291°C for (2b), respectively. The 1H NMR spectrum of compound (2a) revealed a methylene singlet at (d) 4.17 ppm and pyrazole H-3 as a singlet at 8.35 ppm. Two singlet appear, one NH2 group at 8.07-8.10 ppm and other at 11.04 for NH as expected. Based on these data it seemed that a –CH2CN side chain is present and that the cyclization took. By addition of the NH2 in the pyrazole (1) to the CN of the malononitrile to form the amidine intermediate followed by an attack of the newly formed amino group to the CN of (1) to afford the pyrazolopyrimidine (2), as shown in Scheme 1. On the contrary, reaction of compounds (1a, b) with diethylmalonate in ethanolic sodium ethoxide solution gave pyrazolopyrimidines (3a, b). The structure of compounds (3) was confirmed by 1HNMR spectroscopic data. The 1HNMR spectrum of compound (3a) revealed the ester group as a triplet for the CH3 protons at 1.37 ppm and a quartet for the CH2 protons at 4.48 ppm, pyrazole H-3, one NH2 group, and an OH signal at 12.42 ppm (Scheme 1). It has been found that when compound (1a) refluxing in ethanol in the presence of triethylamine afforded a brown crystalline solid of mp 247-249 °C. It was expected that this reaction would give the pyrazolopyrimidine (4a). However, the microanalytical data showed that this product has the molecular formula C_{m}H_{n}N_{p}. Furthermore, the IR spectrum displays an absorption at 3472 and 3312 cm-1, corresponding to NH2 stretching and at 3212 for (NH) cm-1 and no CN absorption. The 1HNMR spectrum reveals two singlets for the amino groups at 5.77 and 5.99 ppm and two singlets for the pyrazole H-3 protons. Structure (4a) was thus suggested for this product. The formation of compound (4a) may be envisaged via initial condensation of the amino group of one molecule of the o-aminonitrile with the cyano group of a second molecule to give an intermediate amidine which then undergoes a second, but intramolecular, amine-nitrile condensation to give the isolated product. A similar result had been established by Taylor and Borror in the formation of (4a) (Scheme 1). Compounds (1b-c) were refluxed under the same reaction conditions to afford (4b-c). Similar cyclizations with other nitriles have been reported. In an attempt to introduce a formyl group at position 3 in pyrazole (1), aminopyrazole (1) was reacted with Vilsmeier reagent (DMF-POCl3) at 70°C for 3h. To the product which was obtained structure (6) was proposed based on the NMR data which indicate the presence of OH group and the pyrazole H-3, the reaction proceeding via the intermediacy of (5) (Scheme 2).

EXPERIMENTAL

General Procedures. Melting points are uncorrected. Microanalyses were carried out in the Microanalytical Centre, Cairo University, Egypt. IR (KBr) spectra were measured on a Karl Zeiss IMP 16 spectrophotometer. 1H-NMR spectra were measured by using Jeol Spectrometer EX-270. Double resonance, HMQC and HMBC experiments were carried out for complete assignment of proton and carbon signals in the NMR spectra, whenever possible. Elemental analyses were obtained on a Leco CHNS-932 instrument. Compound 9 was prepared by a known method.

General procedure for preparation of 2a, b and 3a, b

Compound 5-amino-4-cyano-1H-pyrazole (1a,b), (10 mmol) was added to (10 mmol) malononitrile or diethylmalonate, this mixture was added to 10 mL freshly prepared sodium ethoxide solution prepared by adding 0.5 g sodium metal into absolute ethanol (10 mL) and the mixture was refluxed for 5 h, and left to cool overnight. The solid product so formed was collected by filtration, washed with ethanol and crystallized from ethanol, unless otherwise stated.

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Data:

(4-Amino-1H-pyrazolo [3, 4-d] pyrimidin-6-yl)-acetonitrile (2a): Pale brown crystals (87%) mp 235-237°C (EtOH); Anal. Calcd for: C_{14}H_{17}N_{5} (221.22): C, 65.16; H, 4.54; N, 25.31; O, 21.60%.

(4-Amino-1H-pyrazolo [3, 4-d] phenylpyrazol-6-yl)-acetonitrile (2b): Brown powder (83%) mp 289-291°C (EtOH); Anal. Calcd for: C_{13}H_{10}N_{2}O_{4} (250.26); C, 62.39; H, 4.03; N, 33.58. Found C, 62.43; H, 4.18; N, 33.48. IR (KBr) v: 3462 and 3302, (NH); 2216, (CN) cm⁻¹; δ(C (EtOH)-d₆): 4.17 (s, 2H, CH₂); 8.00 (br s, 1H, NH); 8.07 (br s, 2H, NH₂); 8.10-8.25, 8.35 (s, 1H, H-H3) 11.04 (s, 1H, NH); δ(C (DMSO-d₆): 27.88 (CH₃); 101.13 (C-3a), 118.61 (CN), 135.13 (C-3), 154.78 (C-7a), 159.99 (C-4), 158.55 (C-6).

4-Amino-6-hydroxy-1H-pyrazolo [3, 4-b] pyridine-5-carboxylic acid ethyl ester (3a): White powder (87%) mp 192-194°C (EtOH); Anal. Calcd for: C_{14}H_{13}N_{3}O_{4} (222.21); C, 48.65; H, 4.54; N, 25.21; O, 21.60%.

4-Amino-6-hydroxy-1H-pyrazolo [3, 4-b] pyridine-5-carboxylic acid ethyl ester (3b): White powder (88%) mp 304-305°C (EtOH); Anal. Calcd for: C_{14}H_{13}N_{3}O_{4} (228.30); C, 60.40; H, 4.73; N, 18.78; O, 16.09%.

General procedure for preparation of 4a-c: To a solution of 5-amine-4-cyano- pyrazole (1a-c) (0.1 mol), in ethanol (10mL) and triethylamine (1 mL) was heated under reflux for 7 h and then concentrated under reduced pressure. The solid product so formed was collected by filtration, washed with ethanol and crystallized from EtOH-H₂O.

6-(5-Amino-1H-pyrazol-4-yl)-7H-pyrrolo [2, 3-d] pyrimidin-4-ylamine (4a): Brown powder (87%) mp 247-249°C (EtOH); Anal. Calcd for: C_{15}H_{13}N_{6} (368.40); C, 65.21; H, 4.38; N, 30.42%. Found C, 65.41; H, 4.28; N, 30.62%. IR (KBr) v: 3472 and 3312, (NH); 3122, (NH) cm⁻¹; δ(C (EtOH)-d₆): 5.77 (s, 2H, NH₂-C-5'), 5.99 (s, 2H, NH₂-C-4), 8.02 (s, 1H, H-3'), 8.28 (s, 1H, H-3), 11.13 (s, 2H, NH); δ(C (DMSO-d₆): 104.21 (C-3a), 141.13 (C-3), 146.22 (C-7a), 155.22 (C-5'), 157.94 (C-4), 160.93 (C-6).

6-(5-Amino-1-phenyl-1H-pyrazol-4-yl)-1-phenyl-1H-pyrrolo [3, 4-d] pyrimidin-4-ylamine (4b): Pale brown powder (77%) mp 267-269°C (EtOH); Anal. Calcd for: C_{16}H_{15}N_{6} (384.40); C, 65.21; H, 4.38; N, 30.42%. Found C, 65.41; H, 4.28; N, 30.62%. IR (KBr) v: 3472 and 3312, (NH) cm⁻¹; δ(C (EtOH)-d₆): 5.77 (s, 2H, NH₂-C-5'), 6.01 (s,2H, NH₂-C-4), 7.55-7.62 (m, 2H, Ar-H), 7.88 (appt, 4H, Ar-H), 7.90-7.87 (m, 2H, Ar-H), 8.62 (s, 1H, H-3'), 8.71 (d, 2H, Ar-H), 8.23 (s, 1H, H-3); δ(C (DCI3): 95.04 (C-4'), 102.38 (C-3a), 120.72 (C-2', C-6 or C-2''', C-6'), 122.58 (C-2', C-6 or C-2''', C-6''), 125.46 (C-4 or C-4'), 126.69 (C-4 or C-4'), 128.68 (C-3, C-5 or C-3', C-5'), 127.64 (C-3, C-5 or C-3', C-5'), 131.33 (C-3', 137.25 (C-1 or C-1'), 138.12 (C-1'' or C-1'), 140.03 (C-3), 146.89 (C-7a), 155.19 (C-5'), 157.14 (C-4), 159.42 (C-6).

6-(5-Amino-1-methyl-1H-pyrazol-4-yl)-1-methyl-1H-pyrrolo [3, 4-d] pyrimidin-4-ylamine (4c): Brown powder (82%) mp 265-267°C (EtOH); Anal. Calcd for: C_{17}H_{16}N_{6} (398.46); C, 49.17; H, 4.95; N, 45.87%. Found C, 49.19; H, 5.25; N, 45.56%. IR (KBr) v: 3387 and 3329, (NH) cm⁻¹; δ(C (EtOH)-d₆): 2.45 (3H, s, H₃), 2.46 (3H, s, H₃), 6.74 (s, 2H, NH₂-C-5'), 7.45 (br, 2H, NH₂-C-4), 7.88 (s, 1H, H-3), 8.36 (s, 1H, H-3'); δ(C (DMSO-d₆): 20.99 (CH₃), 20.61 (CH₃), 98.99 (C-4'), 102.99 (C-3a), 121.11 (C-2', C-6'), 123.22 (C-2', C-6'), 129.33 (C-3', C-5'), 130.22 (C-3', C-5'), 135.22 (C-4'), 136.22 (C-1'), 135.22 (C-1'), 139.99 (C-3), 146.99 (C-7a), 157.55 (C-4), 160.91 (C-6).

General procedure for preparation of 6a, b: 10 mmol of (1a, b) was added to phosphoryl chloride (1.915 g, 12.5 mmol) in anhydrous DMF (2.5 mL) was heated under stirring at 70°C for 3 h. Then, the reaction mixture was poured onto ice and treated with aqueous ammonia (pH 8). A white solid separated and it was filtered off, washed with water, dried and recrystallized from an appropriate solvent to afford the products in 80–82% yields.

1H-Pyrazolo [3, 4-d] pyrimidin-4-ol (6a): White powder (75%) mp 301-302°C (EtOH); Anal. Calcd for: C_{15}H_{16}N_{3}O (166.11); C, 44.12; H, 2.96; N, 41.16; O, 11.75%. Found C, 44.29; H, 2.75; N, 41.36; O, 11.85%. IR (KBr) v: 3212 (NH), 3237 (br, OH) cm⁻¹; δ(C (EtOH)-d₆): 8.19 (s, 1H, H-
1-Phenyl-1H-pyrazolo [3, 4-d] pyrimidin-4-ol (6b):

White powder (87%) mp 311-312°C (EtOH); Anal. Calcd for: C_{11}H_{8}N_{4}O (212.21): C, 62.26; H, 3.80; N, 26.40; O, 7.54 %. Found: C, 62.39; H, 3.65; N, 26.56; O, 7.75 %. IR (KBr) v: 3247 (br, OH) cm⁻¹; δ_H (DMSO-d6): 7.41 (t, 1H, H-4), 7.58 (t, 2H, H-3' and H-5'), 8.13 (d, 2H, H-2', H-6'), 8.21 (s, 1H, H-6), 8.39 (s, 1H, H-3), 12.45 (br, 1H, OH).

3. Antibacterial activity

The moiety in pyrazole known for their popular pharmacological activities (19, 20), moiety in pyrazole, both in the form of a substituent or as a fused component, changes its properties and converts it into an altogether new and important heterocyclic derivative. Pyrimidines have attracted particular interest over the last few decades due to the use of such a ring system as the core nucleus in various drugs (19). They are well considering the importance of pyrazolopyrimidine derivatives for their biological activity, it was thought worthwhile to test most of our prepared compounds (2 a,b - 3a,b, 4a-c, 5a-c, 6a,c) for their antibacterial activity against some bacteria namely Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis. The minimum inhibitory concentrations (MIC) were ascertained by the broth dilution method (microdilution using 96-well microplates) (20). The results presented in table 1 showed that 3b, 4c were the most active towards Pseudomonas aeruginosa. We also noticed that adding a CH₂ in the fragment decreases this activity. Compound 4c was the most active against E. coli, Acinetobacter, Pseudomonas Aeruginosa, and Staphlococcus Aureus. The remaining compounds were found to have a slight or moderate activity against the tested organisms.
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Scheme (2)

Table 1 Antibacterial activity of some synthesized compounds

| Compound | Acinetobacter | Pseudomonas Aeruginosa | Escherichia coli | Staphlococcus Aureus | Enterococcus Faecalis |
|----------|---------------|------------------------|------------------|----------------------|-----------------------|
|          | MIC (mg/mL)   |                        |                  |                      |                       |
| 2a       | 1.8           | 1.5                    | 1.5              | 4                    | 3                     |
| 2b       | 4             | 1.5                    | 4                | 2                    | 4                     |
| 3a       | 1.9           | 2                      | 2                | 2                    | 1.8                   |
| 3b       | 1             | 0.8                    | 1                | 1                    | 0.9                   |
| 4a       | 2             | 4                      | 1.5              | 2                    | 4                     |
| 4b       | 1.6           | 1                      | 1.5              | 1                    | 1                     |
| 4c       | 0.9           | 0.8                    | 0.9              | 0.8                  | 1                     |
| 6a       | 3             | 3                      | 2                | 3                    | 4                     |
| 6b       | 1             | 1.1                    | 1                | 1                    | 1.2                   |

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