



Synthesis and antimicrobial activity of Novel Pyrazole Derivatives

HASSAN MOHAMMED AL-GHAMDI*

Department of Chemistry, Faculty of Science, Albaha University, Saudi Arabia.

*Corresponding author E-mail: hassan11220@gmail.com

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ABSTRACT

This work aimed to synthesis some new different heterocyclic compounds based on phenylpyrazole-4-carbaldehyde derivative (1) reacted with cyanoacetohydrazide to afford N'-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-cyanoacetohydrazide (2). It was cyclized by acetyl acetone, and aromatic aldehyde, respectively, to give pyrazolo-pyridone (4) and substituted pyrazolochromene (6 and 8), respectively. Pyrazolo triazole (10) was obtained by coupling of 1-azido-4-fluorobenzene with (2), which was diazotized by 3-(chlorodiazonyl)-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine to give pyrazolo pyridine derivative (11). N,N-Dimethylformamide dimethyl acetal reacted with (2) to afford [(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-cyano-3-(dimethylamino)-acrylohydrazide (13). The compound before compound yield substituted pyrazole-4-carbohydrazide (15). Also, compound (2) reacted with pHNCS in the presence of KOH and furnish compound (22), which preferred over compound (20). All synthesized compounds were established using IR, ¹H-NMR. All synthesized compounds were screened for their antimicrobial activities.

Keywords: Pyrazole, Thiazole, Chromene, Pyridine and Antimicrobial activity.

INTRODUCTION

Pyrazoles have attracted considerable attention for many researchers, due to their broad effects in many different fields¹⁻², as agrochemicals³⁻⁴, antimicrobial⁵, antifungal⁶, anticancer⁷, antidiabetic⁸, anti-inflammatory⁹, antitumor¹⁰, antiviral¹¹, anti-HIV activity¹², and selective enzyme inhibitory activities¹³. Moreover, pyrazolo-chromene derivatives were exhibited powerful antimicrobial and antifungal activities¹⁴⁻¹⁵. Also, pyrazole compounds based on pyridone ring moiety were evaluated against their antibacterial and antifungal activities pyrazolopyridone derivatives exhibit a positive acceptance as

antimicrobial character¹⁶⁻¹⁷. As well as, when introducing pyrazole and thiazole fragments to parent pyrazole were enhancement antimicrobial power¹⁸⁻²¹. The aim of this work is design and synthesis some a new heterocyclic compound based on pyrazole moiety and investigate their biological evaluation as antimicrobial activities.

MATERIALS AND METHODS

All chemicals were obtained from Sigma-Aldrich and used without further purifications. All melting points are uncorrected. IR spectra (KBr) were recorded on FTIR-5300 spectrometer



(λ_{\max} , cm^{-1}). The $^1\text{H-NMR}$ spectra were recorded in (CDCl_3 & DMSO-d_6) on a Bruker Ultra-Shield 850 MHz and reported in (ppm).

Synthesis of N'-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-cyanoaceto hydrazide (2)

A mixture of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (1; 0.02 mol), and 2-cyanoaceto hydrazide (0.02 mol), was refluxed in absolute ethanol (60 ml) for 5 h at 120°C . The reaction mixture was cooled, and the solid product was collected, dried. m.p.: $188-190^\circ\text{C}$, yield: 5.3 g (88%), pale yellow (acetic acid). IR: 1682 (CO), 2263 (CN), 3196 (NH). $^1\text{H-NMR}$: $\delta = 2.55$ (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 7.26 – 7.84 (m, 5H, Ar-H), 7.84 (s, 1H, CH), 9.60 (s, 1H, NH). M.F. $\text{C}_{14}\text{H}_{12}\text{ClN}_5\text{O}$. Calcd: C, 55.73; H, 4.01; Cl, 11.75; N, 23.21; O, 5.30. Found: C, 55.70; H, 3.38; Cl, 11.79; N, 23.26; O, 5.28.

Synthesis of [(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-amino-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (4)

This compound was prepared by a modification to that previously reported method²². A mixture of pyrazole derivative (2; 0.002 mol), acetyl acetone (0.002 mol) in (25 ml) of absolute ethanol and 0.2 ml of piperidine was added. The reaction mixture was heated under reflux for 3 hours. The solid product formed after cooling was filtered, washed with cold methanol, and dried. m.p.: $235-237^\circ\text{C}$, yield: 0.6 g (82%), yellow (benzene). IR: 1652 (C=O), 2216 (CN). $^1\text{H-NMR}$: $\delta = 2.43$ (s, 6H, 2 CH_3), 2.58 (s, 3H, CH_3), 6.09 (s, 1H, CH pyridine), 7.26 – 7.55 (m, 5H, Ar-H) and 8.94 (s, 1H, CH). M.F. $\text{C}_{19}\text{H}_{16}\text{ClN}_5\text{O}$. Calcd: C, 62.38; H, 4.41; Cl, 9.69; N, 19.15; O, 4.37. Found: C, 62.32; H, 4.37; Cl, 9.71; N, 19.18; O, 4.35;

Reaction of pyrazole-2-cyanoaceto hydrazide with aromatic aldehyde derivatives

Compound (2; 0.002 mol) was reacted with aromatic aldehyde derivative (0.002 mol), in absolute ethanol (25 ml) and 0.3 ml of piperidine was added. The reaction mixture was refluxed for 2-3 h at 120°C . The reaction mixture was cooled, and the solid product was collected, and dried.

[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-imino-2H-chromene-3-carbohydrazide (6a)

m.p.: $236-238^\circ\text{C}$, yield: 0.68 g (85%), yellow (ethanol/benzene). IR: 1688 (CO), 3053, 3296

(NH). $^1\text{H-NMR}$: $\delta = 2.61$ (s, 3H, CH_3), 7.12 – 7.78 (m, 10H, Ar-H, chromene-H) 7.79 (s, 1H, CH), 10.01 (s, 1H, NH), 13.49 (s, 1H, NH chromene). M.F. $\text{C}_{21}\text{H}_{16}\text{ClN}_5\text{O}_2$. Calcd: C, 62.16; H, 3.97; Cl, 8.73; N, 17.26; O, 7.88. Found: C, 62.13; H, 3.96; Cl, 8.77; N, 17.25; O, 7.89.

[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-imino-6-methoxy-2H-chromene-3-carbohydrazide (6b)

m.p.: $270-272^\circ\text{C}$, yield: 0.7 g (81%), orange (acetic acid). IR : 1695 (CO), 3055, 3239 (NH). $^1\text{H-NMR}$: $\delta = 2.71$ (s, 3H, CH_3), 3.98 (s, 3H, CH_3 Methoxy), 7.15 – 7.59 (m, 9H, Ar-H, chromene-H), 8.30 (s, 1H, CH), 9.04 (s, 1H, NH), 11.83 (s, 1H, NH chromene-H). M.F. $\text{C}_{22}\text{H}_{16}\text{ClN}_5\text{O}_3$. Calcd: C, 60.62; H, 4.16; Cl, 8.13; N, 16.08; O, 11.01. Found: C, 60.55; H, 4.12; Cl, 8.14; N, 16.10; O, 11.04.

6-bromo-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-imino-2H-chromene-3-carbohydrazide (6c)

m.p.: $248-250^\circ\text{C}$, yield: 0.82 g (86%), yellow (ethanol). IR: 1681 (CO), 3213 (NH). M.F. $\text{C}_{21}\text{H}_{15}\text{BrClN}_5\text{O}_2$. Calcd: C, 52.03; H, 3.12; Br, 16.48; Cl, 7.31; N, 14.45; O, 6.61. Found: C, 51.98; H, 3.11; Br, 16.42; Cl, 7.32; N, 14.43; O, 6.60.

Synthesis of [(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-3-imino-3H-benzo[f]chromene-2-carbohydrazide (8)

The compound (2; 0.002 mol) was cyclized by 2-hydroxy-1-naphthaldehyde (0.002 mol) in absolute ethanol media (20 ml) with catalytic amount of piperidine. The reaction mixture was heated under reflux for 3 h, the solid product formed was filtered, washed with cold methanol, and dried. m.p.: $242-244^\circ\text{C}$, yield: 0.73 g (80%), pale yellow (ethanol/benzene) IR: 1689 (CO), 3049, 3242 (NH). M.F. $\text{C}_{25}\text{H}_{16}\text{ClN}_5\text{O}_2$. Calcd: C, 65.86; H, 3.98; Cl, 7.78; N, 15.36; O, 7.02. Found: C, 65.78; H, 3.94; Cl, 7.76; N, 15.38; O, 7.01.

Synthesis of 5-amino[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-1-(4-fluorophenyl)-1H-1,2,3-triazole-4-carbohydrazide (10)

1-azido-4-fluorobenzene (0.001 mol) was dissolved in absolute ethanol (20 ml). Compound (2; 0.001 mol), in presence of 1.2 g of sodium ethoxide was added to the reaction mixture. Then heated under reflux for about 3 h, the solid

product was filtered, washed with cold ethanol, and dried. m.p.: 280-282°C, yield: 0.33 g (75%), white (1,4-dioxane). IR: 1640 (CO), 3275 (NH), 3422, 4552 (NH₂). ¹H-NMR : δ = 2.51 (s, 3H, CH₃), 6.59 (s, 2H, NH₂), 6.46 – 7.71 (m, 9H, Ar-H), 8.57 (s, 1H, CH), 11.91 (s, 1H, NH). M.F. C₂₀H₁₆ClFN₉O. Calcd : C, 54.74; H, 3.68; Cl, 8.08; F, 4.33; N, 25.50; O, 3.65. Found: C, 54.69; H, 3.64; Cl, 8.10; F, 4.34; N, 25.57; O, 3.62.

Synthesis of {2-[2-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl-ene]hydrazinyl}-N-(4,6-dimethyl-1H-pyrazolo[3,4-b]pyridin-3-yl)-2-oxoacetohydrazonoyl cyanide (11)

A mixture of pyrazole derivative (2; 0.001 mol) and 3-(chlorodiazanyl)-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine (0.001 mol), in pyridine (15 ml). The reaction mixture was stirred at 0°C for 3 h, then poured into ice cold water. The solid product was collected and dried. m.p.: 244-246°C, yield: 0.4 g (85%), green (ethanol/benzene). IR: 1652 (CO), 2217 (CN), 3212, 3262, 3403 (NH). ¹H-NMR: δ = 2.70 (s, 3H, CH₃), 2.92 (s, 3H, CH₃), 3.18 (s, 3H, CH₃), 7.15 – 7.59 (m, 6H, Ar-H), 8.28 (s, 1H, CH), 8.28 (s, 1H, NH), 9.35 and 11.03 (s, 2H, NH). M.F. C₂₂H₁₉ClN₁₀O. Calcd: C, 55.66; H, 4.03; Cl, 7.46; N, 29.48; O, 3.37. Found: C, 55.57; H, 3.98; Cl, 7.47; N, 29.51; O, 3.36.

General procedure for Reaction of pyrazole-2-cyanoacetohydrazide with benzenediazonium chloride derivatives

Pyrazole derivative (2; 0.001 mol) and 1-chloro-2-phenyldiazene derivative (0.001 mol) was dissolved in (15 ml) of pyridine. The reaction mixture was stirred at 0°C for 2 h and poured into ice cold water. The solid product was collected and dried.

2-{2-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl) methyl-ene]hydrazinyl}-2-oxo-N-phenyl acetohydrazonoyl cyanide (12a)

m.p.: 196-197°C, yield: 0.35 g (87%), red (ethanol). IR : 1667 (CO), 2220 (CN), 3042, 3256 (NH). ¹H-NMR : δ = 2.65 (s, 3H, CH₃), 7.18 – 7.99 (m, 10H, Ar-H), 8.16 (s, 1H, CH), 9.17 and 14.14 (s, 2H, NH). M.F. C₂₀H₁₆ClN₇O. Calcd: C, 59.19; H, 3.97; Cl, 8.73; N, 24.17; O, 3.94. Found: C, 59.13; H, 3.94; Cl, 8.75; N, 24.12; O, 3.94.

2-{2-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]hydrazinyl}-N-(4-chlorophenyl)-2-oxoacetohydrazonoyl cyanide (12b)

m.p.: 248-250°C, yield: 0.35 g (81%), yellow (benzene). IR: 1666 (CO), 2220 (CN), 3035, 3256 (NH). M.F. C₂₀H₁₅Cl₂N₇O. Calcd: C, 54.56; H, 3.43; Cl, 16.10; N, 22.28; O, 3.63. Found: C, 54.59; H, 3.37; Cl, 16.12; N, 22.30; O, 3.61.

Synthesis of [(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methyl-ene]-2-cyano-3-(dimethylamino) acrylohydrazide (13)

N,N-dimethylformamide dimethyl acetal (0.001 mol) was added to pyrazole derivative (2; 0.001 mol), in xylene (15 ml). The reaction mixture was refluxed for 4 h at 220°C. The reaction mixture was cooled, and the solid product was collected and dried. m.p.: 230-232°C, yield: 0.32 g (89%), pale yellow (1,4-dioxane). IR: 1674 (CO), 2181 (CN), 3340 (NH). ¹H-NMR: δ = 2.61 (s, 3H, CH₃), 3.39 (s, 3H, CH₃), 3.41 (s, 3H, CH₃), 7.23 – 7.93 (m, 6H, Ar-H and CH), 8.94 (s, 1H, CH), 10.00 (s, 1H, NH). M.F. C₁₇H₁₇ClN₆O. Calcd: C, 57.23; H, 4.80; Cl, 9.94; N, 23.55; O, 4.48. Found: C, 57.16; H, 4.77; Cl, 9.90; N, 23.60; O, 4.43.

Synthesis of 3-amino[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-1H-pyrazole-4-carbohydrazide (15)

A mixture of (13; 0.001 mol), hydrazine hydrate (0.001 mol), in (15 ml) of 1, 4-dioxane. The reaction mixture was heated under reflux for 7 h, the solid product is filtered, washed with cold ethanol, and dried. m.p.: 105-106°C, yield: 0.28 g (87%) white (ethanol). IR: 1597, 1633 (C=N), 3208, 3364 (NH₂). ¹H-NMR: δ = 2.56 (s, 3H, CH₃), 5.40 (s, 2H, NH₂) and 7.19 – 7.51 (m, 7H, Ar-H), 7.67 (s, 1H, CH) and 8.59 (s, 2H, NH). M.F. C₁₅H₁₄ClN₇O. Calcd: C, 52.39; H, 4.08; Cl, 10.34; N, 28.53; O, 4.66. Found: C, 52.35; H, 4.10; Cl, 10.31; N, 28.54; O, 4.62.

General Procedure for [(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-cyano-2-(4-hydroxy-3-phenylthiazol-2(3H)-ylidene)-acetohydrazide (22)

To suspension of potassium hydroxide (0.01 mol) in dry DMF (10 ml) Pyrazole derivative (2; 0.01 mol) was added during stirring, phenyl isothiocyanate (0.01 mol) was dropped slowly to the reaction mixture. After completing of addition, stirring of the reaction mixture was continued for 5 hours.

Then treated with ethyl bromoacetate (0.01 mol) and the stirring was continued at room temperature for 4 hours. The mixture was then triturated with cold H₂O (100 ml) containing HCl (0.1 N). The resultant solid product was collected by filtration and dried. m.p.: 198-199°C, yield: 0.4 g (84%), red (ethanol). IR: 1734 (CO), 2201 (CN), 3254 (NH). ¹H-NMR: δ = 2.58 (s, 3H, CH₃), 4.15 (s, 2H, CH₂), 7.25 – 7.55 (m, 10H, Ar-H), 8.04 (s, 1H, CH), 9.97 (s, 1H, NH). M.F. C₂₃H₁₇ClN₆O₂S. Calcd : C, 57.93; H, 3.59; Cl, 7.43; N, 17.62; O, 6.71; S, 6.72. Found: C, 57.96; H, 3.54; Cl, 7.41; N, 17.64; O, 6.70; S, 6.71.

Antimicrobial activity

The standardized disc – agar diffusion method was followed to determine the activity of the synthesized compounds against the tested microorganisms.

Test Organisms

Cultures of the following microorganism were used in the test: *Gram-positive* bacteria: *Staphylococcus aureus* (ATCC25923) and *Bacillus subtilis* (ATCC6635), *Gram-negative* bacteria: *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028), Yeast: *Candida albicans* (ATCC 10231) and Fungus: *Aspergillus fumigatus*.

Screening for the antimicrobial potential

Preparation of tested compound

The tested compounds were dissolved in dimethyl formamide (DMF) solvent and prepared in two concentrations; 100 and 50 mg/ml and then 10 µl of each preparation was dropped on disk of 6 mm in diameter and the concentrations became 1 and 0.5 mg/disk respectively. In the case of insoluble compounds, the compounds were suspended in DMF and vortexed then processed.

Testing for anti-bacterial and yeast activity

Bacterial cultures were grown in nutrient broth medium at 30°C. After 16 h of growth, each microorganism, at a concentration of 10⁸ cells/mL, was inoculated on the surface of Mueller-Hinton agar plates using sterile cotton swab. Subsequently, uniform size filter paper disks (6 mm in diameter) were impregnated by equal volume (10 µl) from the specific concentration of dissolved compounds and carefully placed on surface of each inoculated plate.

The plates were incubated in the upright position at 36°C for 24 hours. Three replicates were carried out for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of dissolved compound was carried out as negative controls. After incubation, the diameters of the growth inhibition zones formed around the disc were measured with transparent ruler in millimeter, averaged and the mean values were tabulated.

Anti-fungal activity

Active inoculum for experiments were prepared by transferring many loopfuls of spores from the stock cultures to test tubes of sterile distilled water (SDW) that were agitated and diluted with sterile distilled water to achieve optical density corresponding to 2.0x10⁵ spore/ml. inoculum of 0.1% suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min then the same procedure was followed as described above.

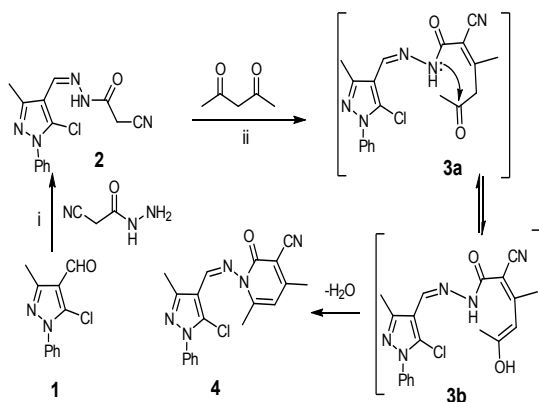
Standard references

The antibiotic chloramphenicol was used as standard reference in the case of *Gram-negative* bacteria, Cephalothin was used as standard reference in the case of *Gram-positive* bacteria and cycloheximide was used as standard reference in the case of yeasts and fungi.

RESULTS AND DISCUSSION

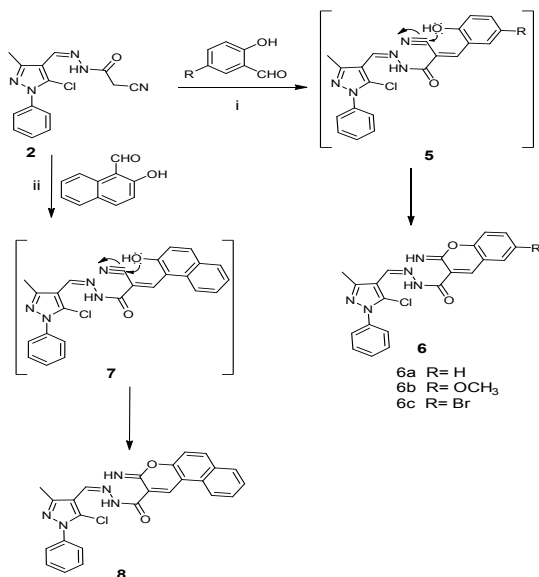
Chemistry

The starting material N'-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-cyanoacetohydrazide (2) was prepared via the reaction of 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (1) with 2-cyanoacetohydrazide as shown in (Scheme 1). The proposed (IR) spectrum of compound showed a prominent peak at 2263 cm⁻¹ assigned for (CN). Whereas the ¹H-NMR (CDCl₃) show signals at δ = 3.85 ppm characteristics for -CH₂- and at 9.60 ppm for NH. when Treatment of N'-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-cyanoacetohydrazide (3) with acetyl acetone afforded the corresponding pyrazolo-pyridone (4). The ¹H-NMR showed a signals at δ = 2.43 ppm characteristics for two methyl and at 6.09 ppm stands for CH in pyridine.



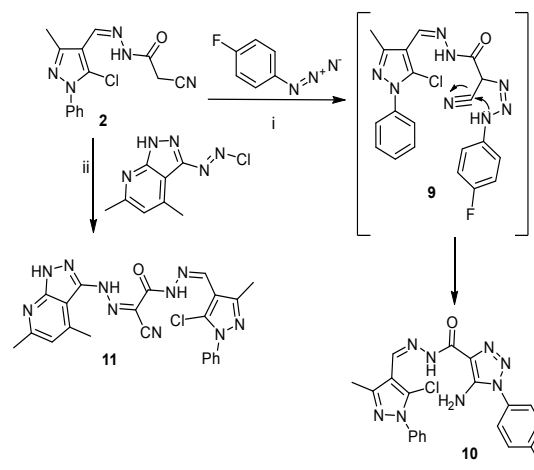
Scheme 1. Reagents and conditions: i-Ethanol, reflux 4-5 h
ii-Ethanol/pip, reflux 3 hours

However, reaction of (2) with salicylaldehyde or its derivatives in ethanol in the presence of piperidine as a catalyst under reflux via elimination of water was afforded the corresponding [(5-chloro-3-methyl-1-phenyl-1H-pyrazolo-4-yl)methylene]-2-imino-2H-chromene-3-carbohydrazide derivatives (6) via the plausible intermediate (5) as shown in (Scheme 2). The disappearance of absorption characteristics band for CN group at 2263 cm^{-1} in 2, and appearance of characteristics for NH at band 3296 cm^{-1} , that supported structure (6a). The other evidence supported (6a), disappearance of characteristic $-\text{CH}_2-$ signal (3.85 ppm), and presence of a characteristic signal for chromene-NH proton at 13.49 ppm. Also, The $^1\text{H-NMR}$ of compound (6b) revealed a characteristic signal for CH_3 of methoxy at 3.89 ppm.



Scheme 2. Reagent and conditions: i-Ethanol/pip, reflux 2-3 h
ii-Ethanol/pip, reflux 3 hours

Moreover, coupling of (2) with 1-azido-4-fluorobenzene in presence of sodium ethoxide as a catalyst, which yielded the corresponding triazole derivative (10). The formation of triazole derivative (10) was assumed to be formed via the acyclic intermediate (9), which then cyclized via addition of amino group to a cyano group (Scheme 2). The infrared (IR) spectrum revealed the disappearance of an absorption band for a characteristic for CN group at 2263 cm^{-1} , in addition to appearance of absorption band characteristics for NH_2 at 3422 and 4552 cm^{-1} . $^1\text{H-NMR}$ showed the disappearance of signal at 3.85 ppm characteristic for $-\text{CH}_2-$ with presence of a characteristic signal at 6.59 ppm for NH_2 group, which supported compound (10).

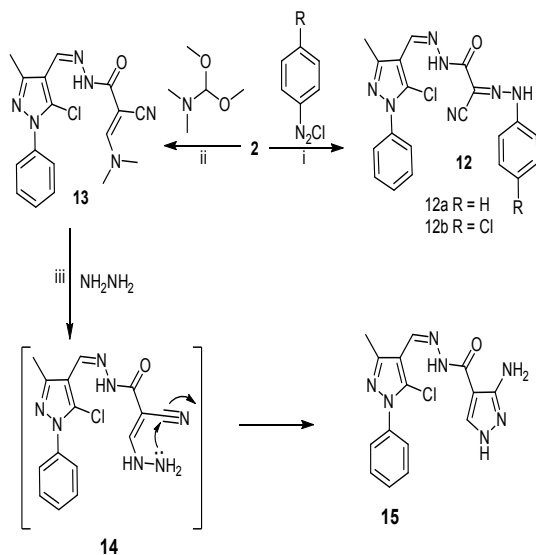


Scheme 3. Reagent and conditions: i-Ethanol/EtONa, reflux 3 h
ii-Pyridine/ 0°C stirrer 3 hours

As well as, reaction of 3-(chlorodiazenyl)-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine in pyridine afforded (11) via elimination of hydrogen chloride. $^1\text{H-NMR}$ (CDCl_3) spectrum of compound (11) revealed the disappearance of characteristic for $-\text{CH}_2$ signal at 3.85 ppm - with appearance of characteristic new $-\text{NH}$ signal at 11.03 ppm.

Also, reaction of benzenediazonium chloride derivative gave (12a, 12b), as shown in (Scheme 3). The infrared (IR) spectrum showed prominent peak at 3256 cm^{-1} assigned for (NH). $^1\text{H-NMR}$ exhibited the disappearance of signal at 3.85 ppm characteristic for $-\text{CH}_2-$ with appearance of signal at 14.14 ppm characteristic for the new NH. Reaction of pyrazole derivative (2) with

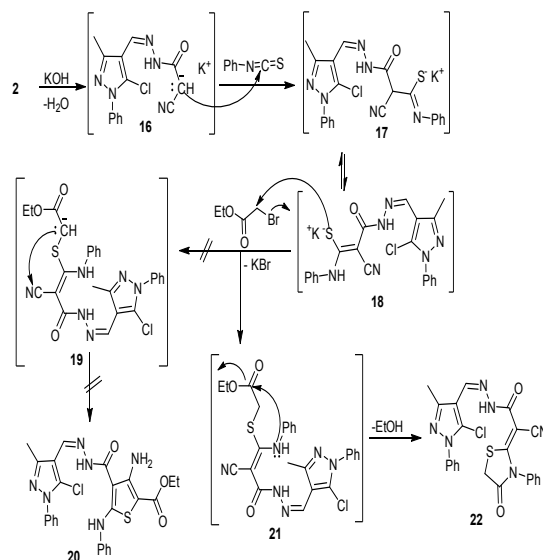
N,N-Dimethylformamide dimethyl acetal in xylene was afforded [(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-cyano-3-(dimethylamino)acrylohydrazide (13) via elimination of two methanol molecules. ¹H-NMR (CDCl₃) spectrum of compound (13) revealed the disappearance of signal at 3.85 ppm characteristic for -CH₂- with absorption signal at 7.23 ppm characteristic for CH.



Scheme 4. Reagent and conditions: i-Pyridine/0°C, stirrer 2 h ii-Xylene, reflux 4 h iii-1,4-Dioxane, reflux 7 hours

The reaction of (13) with hydrazine hydrate in 1,4-dioxane resulted in formation of 3-amino[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-1H-pyrazole-4-carbohydrazide (15) via elimination of dimethylamine, then a cycloaddition reaction, which led to final product, as shown on (Scheme 4). The infrared (IR) spectrum showed presence of characteristics for NH₂ bands at 3208, 3364 cm⁻¹.

Compound (2) reacted with phenyl isothiocyanate in presence of potassium hydroxide afforded an intermediate product of potassium -{2-[-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene] hydrazinyl}-2-cyano-3-oxo-1-(phenylamino)prop-1-ene-1-thiolate (16), which, reacted with ethyl bromoacetate at room temperature to give [(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-2-cyano-2-(4-hydroxy-3-phenylthiazol-2(3H)-ylidene)acetohydrazide (22) as showed in (Scheme 5). It can be concluded that from the obtained



Scheme 5. Reagent and conditions: i-DMF/KOH, stirrer 48 h ii-DMF, stirrer r.t 6 h iii-DMF, stirrer r.t 4 hours

spectral data, an elimination of hydrogen bromide and ethyl alcohol the most plausible mechanistic pathway for prepared compound (22) preferred over compound (20). the proposed mechanism was supported by intense absorption nitrile group band at 2201 cm⁻¹ in IR spectrum, which would be disappeared of compound (20) formed. Whereas the ¹HNMR spectrum showed no peaks for the characteristics signals of ethyl protons.

Biological activity

Agar diffusion method used for evolution antimicrobial potency for tested compounds, using the following microorganism: "Gram-positive bacteria: *Staphylococcus aureus* (ATCC25923) and *Bacillus subtilis* (ATCC6635), Gram-negative bacteria: *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028), Yeast: *Candida albicans* (ATCC 10231) and Fungus: *Aspergillus fumigatus*". From Table 1, the compounds 6b, 6c, 8, 12a, 13 and were found to be inactive against all microorganisms while compounds 2, 3, 6a, 12b and 22 exhibited moderate activity against some microorganisms only and inactive against others. From the study, of the biological activity of these compounds were found that these compounds containing the unit of pyrazole weak or no effect on microbes.

Table 1: antimicrobial activity of the synthesized compounds

Organisms	Gram - positive bacteria						Gram - negative bacteria						Yeasts and Fungi**		
	Staphylococcus aureus (ATCC 25923)		Bacillus subtilis (ATCC 6635)		Salmonella typhimurium (ATCC 14028)		Escherichia coli (ATCC 25922)		Candida albicans (ATCC 10231)		Aspergillus fumigatus				
Concentration	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	1 mg/ml	0.5 mg/ml	
Compounds	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
6:00 AM	11	8	-	-	-	-	-	-	-	-	-	-	-	-	
6 b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
6 c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
12:00 AM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
12 b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Control #	35	26	35	25	36	28	38	27	35	28	37	26	37	26	

CONCLUSION

In this work, we prepared pyrazolo-cyanoacetohydrazide derivative (2) as a new starting material Pyrazolo-pyridone (4), pyrazolo-cheomene (6) and pyrazolo-benzochromene (8) was synthesized via nucleophilic cycloaddition reactions. when substituted aromatic azide was reacted with (2), to give 5-amino-1,2,4-triazole through dipolar cycloaddition reaction mechanism. While, substitution reaction of (2) with diazonium chloride salts afforded (11) and (12). The compound (2) was reacted with phenylisothiocyanate in base medium followed by addition of ethyl bromoacetate afforded the pyrazolothiazole derivative (22). Antimicrobial activity was evaluated for all synthesized compounds the compounds 6b, 6c, 8, 12a, 13 were exhibited inactive potency against all microorganisms while compounds 2, 3, 6a, 12b and 22 were exhibited moderate activity against some microorganisms. Finally, we concluded that, the newly synthesized compounds possess a weak or no effect on microorganisms.

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Conflict Of Interest

The author declare no conflict of interest.

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