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Synthesis, Characterization and Biological Activity of Some Pyrazole-Pyrazolone Derivatives

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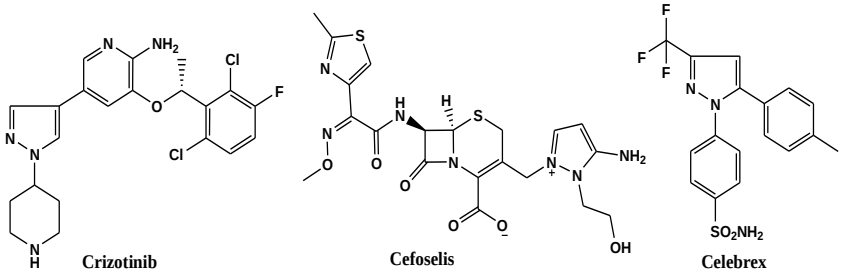
SIX heterocyclic compounds were synthesized based on pyrazole and pyrazolone rings. The chemical structures of the synthesized compounds were characterized by using: elemental analysis, FTIR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra. The compounds were evaluated for their activity against gram +ve, gram –ve bacteria and fungi. The antimicrobial assessment showed the high activity of compounds I and VI.

Keywords: Pyrazole; pyrazolone and Antimicrobial activity.

In the last several decades, pyrazole and pyrazolone derivatives have received considerable attention due to their wide-range of biological activity. Pyrazoles are reported to exhibit antiviral [(1)], antagonist [(2)], antimicrobial [(3)], anti-bacterial[(4)], anticancer[(5)], anti- inflammatory[(5)], analgesic[(6)], anti- prostate cancer[(7)], herbicidal[(8)], acaricidal and insecticidal [(9)] activities and also as anti- Tobacco Mosaic Virus [(10)].

Among pyrazoles, Crizotinib is a drug used asanti-cancer [(11)], Cefoselis is a drug used asantibacterial [(11)] and Celebrex (celecoxib) is a drug used for rheumatoid arthritis and osteoarthritis [(12)].

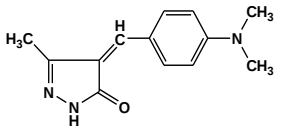


Scheme 1

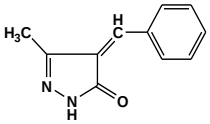
Pyrazolone ring system represents an important class of compounds not only for their theoretical interest but also for their anti-inflammatory, antipyretic[(13)], hypoglycemic agent [(14)], fungicide [(15)], antimicrobial[(16)] and some of them have

been tested as potential cardiovascular drugs [17].

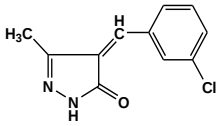
Among pyrazolones, PYZ1, PYZ2, PYZ3 are used as Analgesic, anti-inflammatory and antipyretic [18].



4-[4-N dimethyl amino benzylidine]-3-methyl pyrozolin-5(4H)-one **(PYZ1)**



4-[benzylidine]-3-methyl pyrozolin-5(4H)- one **(PYZ2)**



4-[3-chloro benzylidine]-3-methyl pyrozolin-5(4H)- one **(PYZ3)**

Scheme 2

Experimental

Instrumentation

Melting point:

A digital Stuart SMP3 electric melting point apparatus.

FTIR

Perkin-Elmer 293 spectrophotometer (cm^{-1}) using KBr disks.

^{1}H-NMR spectra

Varian Mercury 400 MHz spectrometer in DMSO-d_6 as a solvent using TMS as an internal standard. Chemical shifts(d) are measured inppmand coupling constants (*J*) in Hz.

Mass spectra

A GC-2010 Shimadzu Gas chromatography instrument mass spectrometer (70 ev)using the electron ionization technique.

Elemental microanalyses

A Perkin-Elmer CHN-2400 analyzerand the microanalyses were within ±0.4% of the theoretical values.

The biological evaluation

The biological evaluation was carried out at Department of Pharmacology, Faculty of Pharmacy, MansouraUniversity, Egypt.

Synthesis

Preparation of 3-amino-1-phenyl- 5-Pyrazolone (I) [19]

In a 25 ml round bottomed flask containing acetic acid 4 ml, phenyl hydrazine (0.01 mole) and ethyl cyanoacetate (0.01 mole) were added. The reaction mixture was refluxed for 8hours. After cooling a viscous product was obtained and the solid obtained after wash with diethyl ether and recrystallized from benzene to give pure product (I), m.p =116^{o}C.

Preparation of 3-amino-1-(2,4-dinitrophenyl)- 5-Pyrazolone (II)[19]

In a 25 ml round bottomed flask containing acetic acid 4 ml, dinitrophenyl hydrazine (0.01 mole) and ethyl cyanoacetate (0.01 mole) were added. The reaction mixture was refluxed for 8hours. After cooling a solid product was obtained and recrystallized from acetic acid to give pure product (II), m.p =185^{o}C.

Preparation of 1H-Pyrazole-3, 5-diamine-4-(2-phenyldiazenyl) (III) [20]

The starting material pyrazole (III) was prepared by literature known procedure using Aniline (0.01 mole) were dissolved in a mixture of concentrated HCl (5mL) and water (5mL) and cooled to 5-10^{o} c in an ice bath. A cold aqueous solution of sodium nitrite (0.01 mole) was added with stirring. The diazonium salt so obtained was filtered into a cold solution of sodium acetate (4g) and malononitrile (0.01 mole) in ethanol (25ml) with stirring for one hour then add hydrazine monohydrate (0.02 mole), a solid product was obtained and recrystallized from ethanol to give pure product (III), m.p =210^{o}C.

Preparation of 1-phenyl Pyrazole-3, 5-diamine, 4-[2-(4-methylphenyl) diazenyl] (IV) [20]

The starting material pyrazole (IV) was prepared by literature known procedure using P-toluidine (0.01 mole) were dissolved in a mixture of concentrated HCl (5mL) and water (5ml) and cooled to 5-10^{o}C in an ice bath. A cold aqueous solution of sodium nitrite (0.01 mole) was added with stirring. The diazonium salt so obtained was filtered into a cold solution of sodium acetate (4g|) and malononitrile (0.01 mole) in ethanol (25ml) with stirring for one hour the add phenyl hydrazine (0.01 mole), a solid product was obtained and recrystallized from ethanol to give pure product (IV), m.p =162^{o}C.

Preparation of 1H- Pyrazole-3, 5-diamine,4-[2-(4-methylphenyl) diazenyl] (V) [20]

The starting material pyrazole (V) was prepared by literature known procedure using P-toluidine (0.01 mole) were dissolved in a mixture of concentrated HCl (5ml) and water (5ml) and cooled to 5-10^{o}C in an ice bath. A cold aqueous solution of sodium nitrite (0.01 mole) was added with stirring. The diazonium salt so obtained was filtered into a cold solution of sodium acetate (4g|) and malononitrile (0.01 mole) in ethanol (25ml) with stirring for one hour the add hydrazine hydrate (0.01 mole), a solid product was obtained recrystallized from ethanol to give pure product (V), m.p =246^{o}C.

Preparation of 5-amino-1, 3–diphenyl-1H-pyrazolecarbonitrile (VI) [21]

The starting material pyrazole (VI) was prepared by literature known procedure using ethanol 7ml and water 7ml, phenyl hydrazine (0.01 mole), malononitrile (0.01 mole) and benzaldehyde (0.01 mole). The mixture was stirred at room temperature for 30 minutes. After the reaction completed the solid product was formed, collected the solid and recrystallization by ethanol to give pure product (VI), m.p =146^{o}C.

Results and Discussion

Structure of the synthesized compounds

The chemical structures of the synthesized were confirmed using different spectroscopic analysis.

Compound (I): Infrared (KBr) υmax 3286, 3233 (NH2), 3030 (CH2), 2939, 2857 CH (aromatic), 1644 (C=O), 1597 (C=N) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ (ppm):6.67-7.48 (m, 5H, Ar-H), 1.89 (s, 2H, CH_2), 7.5, 9.5 (s, 2H, NH_2 exchangeable).^{13}C NMR (DMSO-d_6) δ (ppm):20.5 (CH_2), 111.7, 118.3, 128.7, 149.3(aromatic C), 168.9 (C-NH_2), 175.1 (C=O). Mass spectrum showed molecular peak at m/z 175(6.12%).Anal. Calcd for $C_9H_9N_3O$ (175.18): C, 61.70; H, 5.18; N, 23.99. Found: C, 61.66; H, 5.20; N, 24.00.

Compound (II): Infrared (KBr) υmax 3338 (NH_2), 3182, 3115 (CH_2), 3015 (CH aromatic), 1662 (C=O), 1619 (C=N), 1587, 1369 (NO_2) cm^{-1}.^{1}H NMR (DMSO-d_6) δ (ppm): 2 (s, 2H, CH_2), 7.21-8.85 (m, 5H, Ar-H), 9.98, 10.35 (s, 2H, NH_2 exchangeable).^{13}C NMR (DMSO-d_6) δ (ppm):20.5 (CH_2), 115.4, 123, 129.5, 136.5 (aromatic C), 148 (C-NH_2), 168 (C=O).Mass spectrum showed molecular peak at m/z 265(2%). Anal. Calcd for $C_9H_7N_5O_5$ (265.18): C, 40.76; H, 2.66; N, 26.41. Found: C, 40.80; H, 2.50; N, 26.38.

Compound (III): Infrared (KBr) υmax 3392 (NH_2), 3296 (NH) 3015 (CH aromatic), 1614 (C=N), 1567 (N=N) cm^{-1}.^{1}H NMR (DMSO-d_6) δ (ppm): 7.17-7.67 (m, 5H, Ar-H), 3.1, 3.3, 5.8, 6 (5H, $2NH_2$, NH exchangeable).Mass spectrum showed molecular peak at m/z 202(26.27%). Anal. Calcd for $C_9H_{10}N_6$ (202.22): C, 53.46; H, 4.98; N, 41.56. Found: C, 53.40; H, 5.00; N, 41.59.

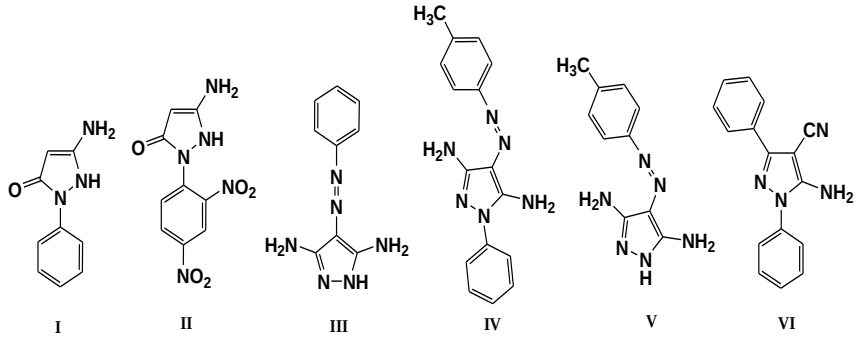
Compound (IV): Infrared (KBr) υmax 3442, 3365, 3321, 3274 (NH_2), 3055, 3023 (CH_3), 2910 (CH aromatic), 1619 (C=N), 1559 (N=N) cm^{-1}.^{1}H NMR (DMSO-d_6) δ (ppm): 2.34 (s, 3H, CH_3), 7.21-7.67 (m, 10H, Ar-H), 5.83, 9.91 (s, 4H, NH_2 exchangeable).^{13}C NMR (DMSO-d_6) δ (ppm):20.68 (CH_3), 114, 120, 121, 129, 136, 138 (aromatic C), 151.2 (C-NH_2).Mass spectrum showed molecular peak at m/z 292(6.15%). Anal. Calcd for $C_{16}H_{16}N_6$ (292.34): C, 65.74; H, 5.52; N, 28.75. Found: C, 65.80; H, 5.42; N, 28.78.

Compound (V): Infrared (KBr) υmax 3395 (NH_2), 3299 (NH), 3055, 3186 (CH_3), 2914 (CH aromatic), 1616 (C=N), 1564 (N=N) cm^{-1}.^{1}H NMR (DMSO-d_6) δ (ppm): 2.32 (s, 3H, CH_3), 7.17-7.57 (m, 4H, Ar-H), 5.99 (4H, NH_2 exchangeable), 10.62(s, H, NH exchangeable).^{13}C NMR (DMSO-d6) δ (ppm):20.65 (CH3), 113, 120, 129, 135 (aromatic C), 151.4 (C-NH_2).Mass spectrum showed molecular peak at m/z 216 (1.55%). Anal. Calcd for $C_{10}H_{12}N_6$ (216.24): C, 55.54; H, 5.59; N, 38.86. Found: C, 55.55; H, 6.00; N, 38.45.

Compound (VI): Infrared (KBr) υmax 3438, 3311 (NH_2), 3053, 3025 (CH aromatic), 3260(CN), 1597 (C=N) cm^{-1}.^{1}H NMR (DMSO-d_6) δ (ppm): 6.72-7.86 (m, 5H, Ar-H), 10.303 (s, 2H, NH_2 exchangeable). Mass spectrum showed

molecular peak at m/z 260 (3.11%). Anal. Calcd for $C_{16}H_{12}N_4$ (260.39): C, 73.83; H, 4.65; N, 21.52. Found: C, 73.90; H, 4.44; N, 21.66.

The results of these analyses confirmed their chemical structures as represented in Schemes 3-1& Fig. 1-4.



Scheme 3. Chemical structure of the synthesized compounds.

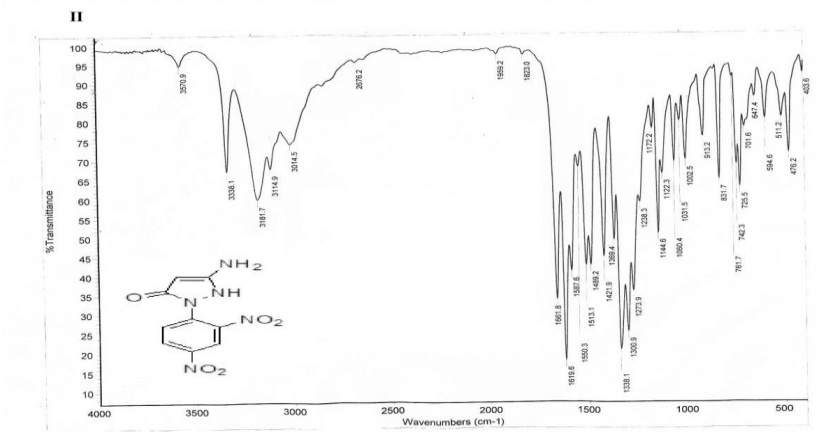


Fig. 1.

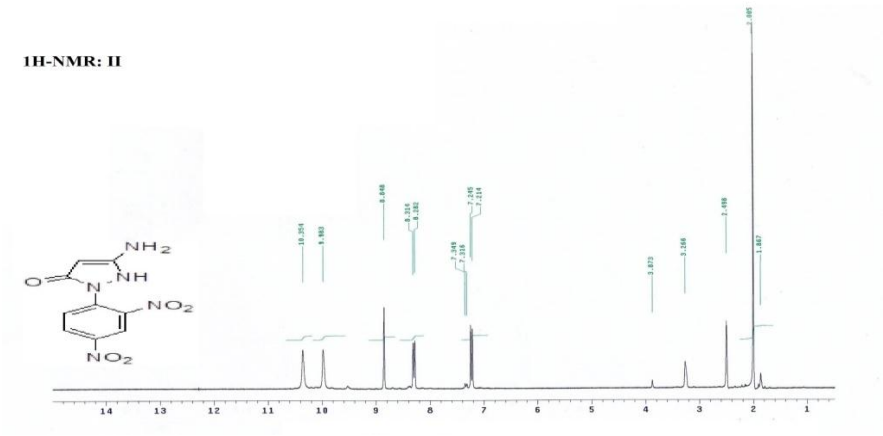


Fig. 2.

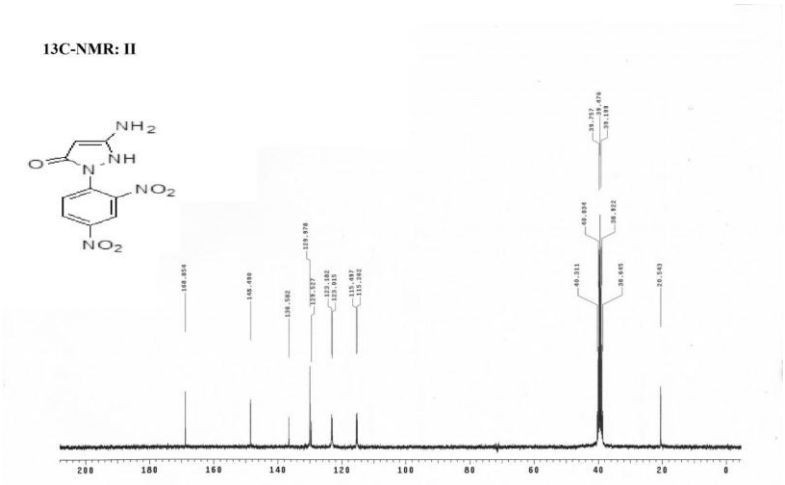


Fig. 3.

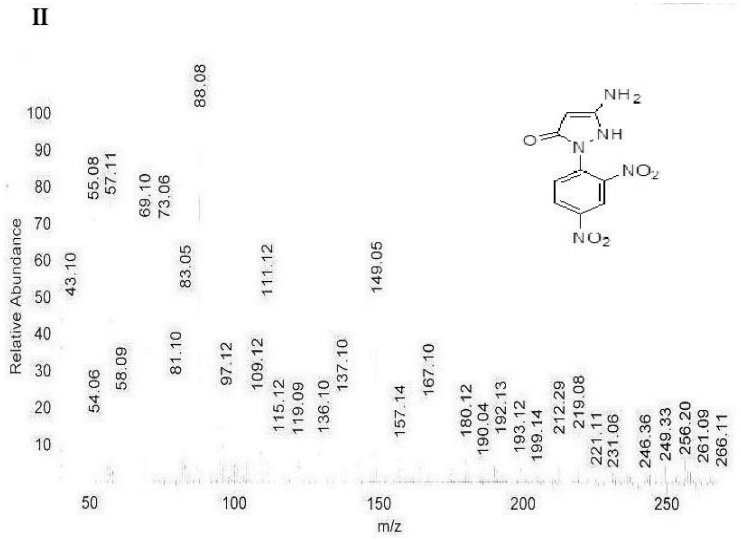


Fig. 4.

Biological assessment

Antimicrobial activity

The antimicrobial activity of the tested compounds was determined using the disc diffusion technique [22, 23] by preparing discs containing 1.9 – 1000 µg/ml of each compound againstgram positive *Staphylococcus aureus* , *Bacillus subtilis* and gram negative *Escherichia coli* , *Pseudomonas aeuroginosa* . The anti-fungal activities of the compounds were tested against two fungi *Candida albicans* , *Aspergillus flavus*. Different dilutions were prepared. The plates were incubated at 37^{o}C for 24 hr. for bacteria and at 28 ^{o}C for 72 hr for fungi. The standard antibiotic ampicillin and antifungal colitrimazolewere used as references. At the end of the incubation period, the minimum inhibitory concentrations (MIC) values were recorded (Table 1) 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 conditions.

The results demonstrate that tested fungi were more sensitive to all compounds compared with bacteria. The most active compounds against fungi were I and IV. While for bacteriaI and IV for Gram negative and for Iand IV Gram positive. In addition, Gram negative bacteria were more sensitive to the compounds compared with Gram positive ones.

TABLE 1. Antimicrobial and Antimycotic Activities in terms of MIC (µg/ml).

Compounds	*A. fumigatus*	*C. albicans*	*S. pneumoniae*	*B. subtlis*	*P. aeruginosa*	*E. Coli*
I	**21.3**	NA	21.4	23.5	19.3	22.4
II	19.3	NA	19.3	21.3	17.4	20.6
III	20.6	NA	20.3	33.5	17.9	22.1
IV	NA	NA	NA	NA	NA	NA
V	15.2	NA	16.4	18.1	NA	15.3
VI	22.5	NA	23.2	25.3	19.6	23.1
Slandered	23.7	25.4	23.8	32.4	20.8	23.4

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تحضير وتشخيص النشاط البيولوجي لبعض مشتقات البيرازول والبيرازولون

جلال حسني سيد[1] ،نبيل نجم[2]، محمد عماد عزب[1] و كيرلس اكرام انور[1]

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تم تحضير ست مركبات تعتمد علي حلاقات البيرازول والبيرازولين وأكد البنيان الكيميائي للمركبات المحضرة بإستخدام التحليل الطيفي بالأشعة تحت الحمراء (FTIR) والرنين النووي المغناطيسي البوتوني (^{1}H-NMR) . ونم تعيين النشاط ضد الكائنات الدقيقة للمركبات المحضرة بما في ذلك: البكتريا والفطريات بإستخدام التقنيات للتثبيط.يبين تقييم مضادات الميكروبات للمركبات المحضرة علي النشاط البيولوجي العالي للمركبات Iو VI.