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Research Article

A CATALYTIC METHOD FOR THE SYNTHESIS OF PYRAZOLONE DERIVATIVES USING HETEROPOLYACIDS AND STUDY OF THE ANTI-BACTERIAL ACTIVITY

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ABSTRACT

A series of pyrazolone derivative 3 was synthesized by following two different procedures: The reaction of 3-acetyldihydrofuran-2(3*H*)-one 1 and thiosemicarbazide derivatives 2 in ethanol without catalyst or from an efficient and improved procedure using Keggin-type heteropolyacid (HPAs). In this case excellent yields and short reaction times were obtained.

The synthesized compounds 3 were screened for their in vitro anti-bacterial activity against 4 bacterial strains.

Keywords: pyrazole; furanone; thiosemicarbazide; heteropolyacid; antibacterial activity.

INTRODUCTION

In spite of the large number of antibiotics available for medical use, the emergence of new antibiotic resistant bacterials trains in the last decades is an alarming problem that seriously affects human health. As a result, search for new antibacterial agents remain as an important and challenging task for medicinal chemists.

Thus the pyrazolone nucleus has attracted much interest in the development of pharmacologically active compounds. In order to enhance potential antimicrobial activity of the pyrazolones derivatives, various research teams varied the structures of the groups with more complex moiety.

The pyrazolone nucleus is a prominent structural moiety and plays an essential role in biologically active compounds[1-3]. Many of the therapeutically useful compounds belong to pyrazoles[4]. Pyrazolone derivatives are of particular importance in pharmaceutical chemistry due to their numerous applications as analgesic[5], antipyretic[6], anti-inflammatory[7,8], antibacterial[9], antifungal[10], antidepressant[11], antitumor agent[12] and anti-proliferative agents[13,14], beside their capability to exert remarkable anticancer effects through inhibiting different types of enzymes that play important roles in cell division[15-17]. In agriculture, they are used as pesticides[18], insecticides[19] and some of them are applied as dyestuffs[20]. Therefore, the synthesis and selective functionalization of pyrazoles have been focus of active research area over the years.

There have been a number of practically important routes to synthesis of pyrazole; the most important methods are the reaction between hydrazines derivatives with 1,3-diketones or their synthetic equivalents (β-oxoesters, β-oxonitriles, β-dialkylamino- or β-alkoxyvinyl ketones) known as the Knorr pyrazole synthesis[21]. Pyrazoles were obtained *via* the Vilsmeier-Haack reaction from the reaction of aryl ketone with phenylhydrazine[22] or via Ugi reaction[23].

In other hand, the use of heteropoly acids (HPAs) in different areas of the organic chemistry has now reached significant levels, not only for the possibility to perform environmentally benign synthesis, but for the good yields[20,24]. HPAs have several advantages as catalysts which make them economically and environmentally feasible. They are stronger acids than homogeneous acid catalysts such as sulfuric acid or ion exchange resins. The use of HPAs as a catalyst is important in the development of clean technologies, since it avoids the drawbacks of environmental pollution and prevents corrosion of the conventional technologies.

In continuation of our previous work concerning the preparation of biologically active heterocycles[25], we wish to describe a novel synthetic route to substituted pyrazolones bearing at N_1 position a carbothioamide functionality. Expecting an enhancement of the antimicrobial potential. the antibacterial activity of pyrazolones 3 was evaluated.

MATERIALS AND METHODS

Melting points were measured on a Buchi 512 apparatus and were uncorrected. FTIR were taken in KBr on a Perkin –Elmer spectrometer. The ^{1}H NMR spectra (250 and 300 MHz) and ^{13}C NMR (63 MHz) were run on a Bruker spectrometer in DMSO using tetramethyl silane as internal standard. The ESI/MS mass spectra were recorded on a Nermag R-10-10C at 70 ev. Chemicals were purchased from Aldrich and Fluka.

General procedure for the synthesis of pyrazolones 3a-c

Method a: A mixture of compounds 1 (10m mol) and the appropriate Isothiocyanates 2 (10 m mol) was refluxed in ethanol for appropriate time. The solid obtained was recrystallized from ethanol.

Method B

To a solution of products 1 (10m mol) and the appropriate isothiocyanates 2 was added 1% (1 µmol, 2.10^{-3} g) of Keggin catalyst ($H_4SiW_{12}O_{14}$, nH_2O) in ethanol. The mixed solution was heated at reflux under magnetic stirring. After cooling to room temperature, the white solid was collected by filtration, and latter recrystallized in ethanol.

Characterization data for compound 3a

(2-[1-(2-oxodihydrofuran-3(2*H*)-ylidene) ethyl] hydrazinecarbothioamide) : mp = 176–178 °C.

^{1}H NMR (300 MHz, DMSO-d6) : 2.15(s, 3H, CH_3) ; 2.27(t, 2H, CCH_2, J=6.85Hz) ; 3.40(t, 2H, OCH_2, J=6Hz).

^{13}C NMR (75 MHz, DMSO-d6) : 11.39, CH_3; 25.66, CH_2; 39.95, C-C=O; 60.02, CH_2; 151.1, C-C-N; 162.8, C=O; 176.39, C=S

ESI/MS ($M+Na^+$) $C_7H_{11}N_3O_2SNa$: m/z = 224.0497.

Characterization data for compound 3b (*N*-methyl-4-(2-hydroxyethyl)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide) : mp = 156–158 °C.

^{1}H NMR (300 MHz, DMSO-d6) : 2.02 (50%) and 2.16(50%) (s, 3H, CH_3) ; 2.31(t, 2H, CCH_2, J=6Hz) ; 2.86 and 3.09(d, 3H, NCH3) ; 3.40(t, 2H, OCH_2,J=6HZ)

^{13}C NMR (75 MHz, DMSO-d6) : 11.41 (CH_3), 14.05 and 14.28 (CH_3), 25.87(CH_2), 40.20(CH_2), 61.93(CH_2) (CH_2), 98.35(C-C=O),150.84(C-N), 160.34(C=O), 174.77(C=S).

ESI/MS ($M+Na^+$) $C_8H_{13}N_3O_2SNa$: m/z = 238.0627

Characterization data for compound 3c (*N*-ethyl-4-(2-hydroxyethyl)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide) : mp = 178–180 °C.

^{1}H NMR (300 MHz, DMSO-d6) : 1.18 (t, J = 6 Hz, 3H), 2.03 (50%) and 2.16 (50%) (s, 3H); 2.34 (t, J = 7 Hz, 2H), 3.39 (q, J = 7 Hz, 2H), 3.60 (q, J = 7 Hz, 2H).

^{13}C NMR (75 MHz, DMSO-d6) : 11.41(CH_3), 14.05(CH_3), 25.87(CH_2), 40.20(CH_2), 61.93(CH_2) (CH_2), 98.35(C-C=O),150.84(C-N), 160.34(C=O), 174.77(C=S).

ESI/MS ($M+Na^+$) $C_9H_{15}N_3O_2SNa$: m/z = 252.0779.

Antibacterial activity

The synthesized compounds were screened for their in vitro antibacterial activity against Gram positive (*Staphylococcusaureus*) and three Gram negative (*Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa*) bacterial strains.

Disc diffusion test

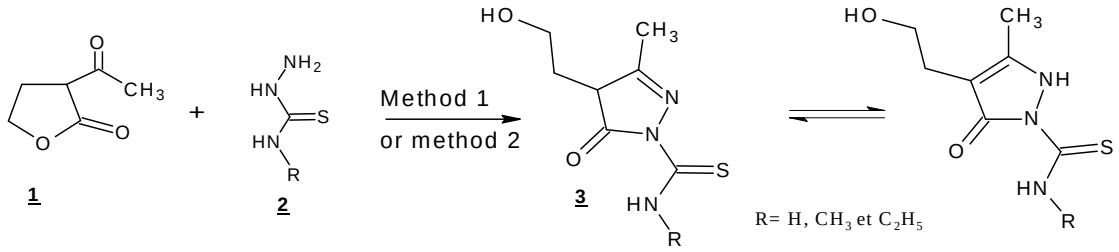
The compounds were dissolved in dimethylsulfoxide (DMSO) and 30 µg of each compound at 100 µg/ml were tested using a filter paper disc diffusion method with Mueller-Hinton agar media. For each experiment, DMSO alone was used as a control and found inactive in the culture medium. Ampicillin was used as reference standard and dissolved in DMSO to get a final concentration of (100 µg/ml). Growth inhibition was examined after 24 h of incubation at 37 °C; antibacterial activity was determined by measuring the zone of inhibition (ZOI) in millimeters around the each disk.

Minimum inhibition concentration

The Microbroth dilution method[26] was used to determine the minimum inhibitory concentration (MIC) of the compounds and reference antibiotic (RA) on a given microorganism. MIC is defined as the lowest concentration of an antimicrobial agent that inhibits any observable growth of the microorganism. Mueller Hinton broth was used as nutrient media to grow. Inoculum size for test strain was adjusted to 108 CFU (Colony Forming Unit) per milliliter by comparing the turbidity. DMSO was used to dilute to get desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. After adding the inoculums and incubating, the lowest concentration of the compound that completely inhibits the growth of the microorganism is identified as the MIC. The preparation of the culture medium (Mueller Hinton broth), inoculates, and compounds is the same as previously described [26]. The studies were tested on the 4 species of the bacteria while all compounds were in deferent's concentration (100, 50, 25, 12.5 and 6.25 µg/mL).

RESULTS AND DISCUSSION

The targeted *N*-R-4-(2-hydroxyethyl)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide 3 were obtained in 50-70 % yield by refluxing furanone 1 with thiosemicarbazides derivatives 2 in ethanol media for 13- 18 h or by use heteropoly acid ($H_4SiW_{12}O_{14}$, nH_2O) as catalyst. In this case the products 3 were obtained in 75-80 % yield by refluxing in ethanol for 5- 8 h (Scheme 1).



Sheme 1

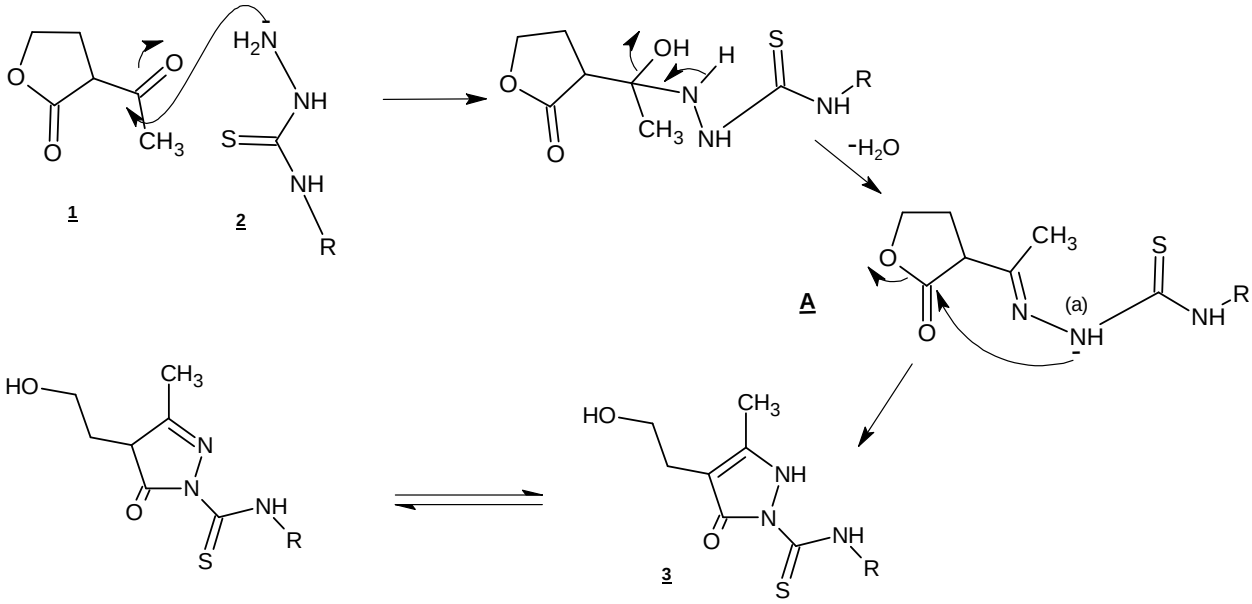
The structure of compound 3 was established on the basis of its spectroscopic data.

The HRESI mass spectrum of 3 gave *a* quasi -molecular ion $(M+Na)^+$ peak at m/z = 252 . The IR spectrum of compound 3 showed absorption at 3430 cm^{-1} and 1615 cm^{-1} due to the OH and C=O stretching respectively. The proton nuclear magnetic resonance spectrum of 3b and 3c in DMSO-d6 revealed two signals due to methyl protons. Spectrum of 3b showed two signals at δ 2.02 and 2.16 ppm respectively, and the integral ratio of the two signals was 1:1 suggesting existence of the tautomeric equilibrium as showed in scheme 1. On the other hand, the ^{13}C- NMR also exhibited methyl carbon signals respectively at δ 14.05 and 14.28 ppm in accord with this tautomeric equilibrium.

Table 1: physical data of derivatives 3

product	R	mp (°C)	Conventional heating (Without catalyze)		$H_4SiW_{12}O_{14}$, nH_2O catalyze	
			Time(h)	Yields (%)	Time(h)	Yields (%)
3a	H	178-180	13h00	70	05h00	85
3b	CH_3	156-158	15h00	55	06h00	75
3c	C_2H_5	176-178	18h00	50	08h00	80

In scheme 2 we suggest plausible mechanism to account for the formation of compound 3. Their formation may proceed via the imine intermediate A formed in the first step as the result of attack of the amino group (2) upon the carbonyl of acetyl fragment (1) followed by dehydration. Attack of the more nucleophilic nitrogen N_a of thiohydrazide moiety on the carbonyl group of the intermediate A lead to furanone ring opening and formation of a desired substituted pyrazolone 3.



Scheme 2

Antibacterial activity

All of the compounds (Table 2) exhibited different antibacterial activities for all four bacterial strains. None of the tested pyrazolones 3a–3c had any effect on the growth of the Gram-negative bacteria *Escherichia coli* (EC), in contrast they showed moderate activity against Gram-positive bacteria *S. aureus* (SA), Gram-negative bacteria *K. pneumoniae* (KP) and P. *aeruginosa*(PA). Compound 3b (ZOI[KP] = 15 mm) and 3c (ZOI[PA] = 15 mm),were identified as the most potent antibacterial agents and have greater activity against pathogenic bacteria than the standard antibiotic drug, Ampicillin. Moreover, derivative 3c (ZOI = 13 mm) inhibits the growth of Gram-positive bacteria *S. aureus.*

Table 2: Zone of inhibition (in mm) for pyrazolones 3a-c against tested bacterial strains at a concentration of 100 µg /mL.

Compounds	*E. coli* *ATCC4157*	*K.pneumoniae* ATCC 27852	*S. aureus* ATCC6538	*P. aeruginosa* ATCC9027
3a	06	10	11	10
3b	06	15	10	12
3c	06	10	13	15
Ampicillin	16	06	25	10

The minimum inhibitory concentration (MIC) values for derivatives 3a–3c were also determined against the four bacterial strains by the solid microdilution method [26]. Concentrations of derivatives at 6.25, 12.5, 25, 50 and 100 µg/mL were prepared in dimethylsulfoxyde (DMSO) solution.

The minimum concentration at which no growth was observed and taken as the MIC value, the result is summarized in Table 3.

Table 3: Minimum inhibitory concentration (MIC in µg/ mL) values for pyrazolones 3a-c against tested bacterial strains

Compounds	*E. coli* *ATCC4157*	*K.pneumoniae* ATCC 27852	*S. aureus* ATCC6538	*P. aeruginosa* ATCC9027
3a	>100	100	12.5	12.5
3b	>100	6.25	12.5	6.25
3c	>100	100	6.25	6.25

Note: control treatment (DMSO) had no inhibitory effect on any of the test bacteria

All Compounds 3 possessed comparable or superior activity as Ampiciline against *P. aeruginosa* with a MIC ≤12.5 µg/mL and with a diameters of inhibition zone ≥ 10 mm. Compounds 3 showed significant activity against *S. aureus* (with a MIC ≤12.5 µg/mL and diameters of inhibition zone 10-13 mm). Compound 3b possessed a good antibacterial activity against *Klebsiella pneumoniaeles* (ZI = 15 mm and with a MIC = 6.25 µg/mL).

However, none of the compounds was superior to the standards used against *S. aureus* and against *Klebsiella pneumoniaeles*. *Escherichia coli* is the most resistant bacteria to these compounds 3 followed by *Klebsiella pneumoniaeles*.

CONCLUSION

Pyrazolones derivatives 3 were prepared by reaction of 3-acetylfurane (1) with isothiocyanates. The heterocyclization reaction was carried without catalyst and using heteropolyacid ($H_4SiW_{12}O_{14}$, nH_2O) in ethanol. The use of Keggin catalyst shows the shortest reaction times and brings rate increase of the yield.

The series of compounds 3a-c were evaluated for their anti-bacterial activity. The derivatives 3, exhibits highly anti-bacterial activity against Pseudomonas aeruginosa.

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