New 4-(substituted)-pyrazolone. Part 1: Microwave-assisted synthesis and antibacterial activity of novel 4-substituted azetidinone derivatives of pyrazolone

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ABSTRACT: In the present research work, the main motto was to develop novel chemical entities of pyrazolone. The starting material 3-methyl-1-(2,4-dinitrophenyl)-1H-pyrazol-5(4H)-one (1) was synthesized in high yields according to reported method using by the treatment of ethyl aetoacetate with appropriate 1-(2,4 -dinitro) phenylhydrazine. Ethyl 2-(4,5-dihydro-3-methyl-1-(2,4-dinitrophenyl)-5-oxo-1H-pyrazol-4-yl)acetate (2) was synthesized by reaction of ethyl chloroacetate with (1). 2-(4,5-dihydro-3-methyl-1-(2,4-dinitrophenyl)-5-oxo-1H-pyrazol-4-yl)acetohydrazide (3) prepared by reaction of hydrazine hydrate with (2) in presence of solvent methanol. The microwave assisted reaction of (3) with different substituted aldehyde in presence of ethanol gives formation of N-(substituted benzylidenyl)-[2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)acetohydrazide] (4a-4e). The microwave assisted reaction of (4a-4e) with chloroacetyl chloride and triethylamine in presence of DMF as solvent gives formation of 2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)-acetamide-(3-chloro-4-substituted phenylazetidin-2-one) (RSa-RSe). The products, characterized on the basis of spectral data, have shown moderate to good antibacterial activity against bacteria. The newly synthesized compounds were screened for antibacterial activity.

KEYWORDS: Pyrazolone; azetidin-2-one; antibacterial activity.

Introduction

Pyrazolones (2-pyrazolin-5-ones, systematic name: 2,4-dihydro-3H-pyrazol-3-ones) are valuable synthons in the construction of various pyrazole-based molecules including, amongst others, metal extracting agents, dyestuffs, photographic developers, and agrochemicals. Furthermore, pyrazolones and derivatifs thereof form the core of many biologically active compounds, comprising also many common drug molecules. Pyrazolone moiety, possess anticancer, antiviral, antitubercular, antihyperlipidemic, antidepressant, antooxidant, antibacterial, anti-HIV, anti-inflammatory, analgesic, antipyretic activities.

The tautomerism of pyrazolones (structure A, B, C) is an old problem of pyrazole chemistry and thus it has been a subject of a considerable number of studies.

Azetidinones are a very important class of compounds possessing a wide range of biological activities such as antimicrobial, antiinflammatory, anti-convulsant, anticancer, anti-elastase, anti-viral, and anti-HCMV activities.

Results and Discussion

The antibacterial activity of compounds (4a-4e) and (RSa-RSe) are reported in (Table 2 and 3) respectively.
Compounds with para N,N-dimethyl amino, para hydroxy, para methyl substitution on phenyl ring attached to 4th position of azetidin-2-one ring such as RSa, Rsb, RSe revealed good antibacterial activity as compared to compounds RSc, Rsd which contain para nitro and para chloro substitution on same ring.

Under identical conditions the standard antibiotic amoxicillin (30 µg/ml) exhibited a zone of inhibition of 18 and 19 mm against Escherichia coli and Bacillus subtilis respectively. The antibacterial activity of compound Rsb is almost more than that of ampicillin against Escherichia coli.

Experimental Section

All melting points were determined in Micro-Controller Melting point apparatus Chemi line CL-725 and are uncorrected. TLC was performed on Silica Gel Plates TLC plates and visualized under UV light (UV Chamber: Rolex UV cabinet Model No. R/340/OC) or iodine chamber. All FT-IR spectra were recorded on Shimadzu 8400-S FT-IR spectrophotometer using KBR. Microwave reactions were carried out using Microwave oven (LG Model No. MG-396WA, 1000W, microwave frequency 2450 MHz). The 1H NMR spectra were recorded in a Bruker spectrophotometer AMX-400 (400 MHz), Bruker Optik (Germany) in CDCl3 using TMS as an internal standard. All Mass spectra were recorded using a Jeol-D-300 Mass spectrophotometer (70 ev), SHIMADZU (Japan) by LCMS-2010A.

### Table 1 Physical data of compounds (4a-4e) and (RSa-RSe).

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<tr>
<th>Compound code</th>
<th>-R</th>
<th>Molecular formula</th>
<th>MW irradiation time (min)</th>
<th>Yield (%)</th>
<th>M. P. (°C)</th>
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<td>-4-N-(CH₃)₂</td>
<td>C₂₁H₂₀N₇O₆</td>
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<td>-4-NO₂</td>
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<td>183</td>
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<td>-4-CH₃</td>
<td>C₂₀H₁₈N₆O₆</td>
<td>6</td>
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<td>-4-N-(CH₃)₂</td>
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### Table 2 Antibacterial activity of compounds# (4a-4e) and (RSa-RSe).

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<tr>
<td></td>
<td>10 µg/ml</td>
<td>20 µg/ml</td>
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<td>Ampicillin</td>
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# = zone of inhibition in mm
Procedure for the preparation of 3-methyl-1-(2,4-dinitrophenyl)-1H-pyrazol-5(4H)-one$^1$ (1)

Mix together 50 g (49 ml, 0.384 mole) of ethyl acetoacetate and 40 g (36.5 ml, 0.37 mole) of 2,4-dinitrophenyl hydrazine in a RBF. Heat the mixture on a boiling water bath in the fume cupboard for about 2 hours. Allow heavy reddish syrup to cool somewhat, add about 100 ml of ether and stirred mixture vigorously. The syrup which insoluble in ether will solidify within 15 minutes. Filter the solid at the pump and it with ether to remove colored impurities. Recrystallized from ethanol.

Scheme

\[ \text{a} = \text{Chloroethyl acetate, 1,4-dioxane} \]
\[ \text{b} = \text{NH}_2\text{NH}_2, \text{H}_2\text{O}, \text{MeOH, Reflux 20 h} \]
\[ \text{c} = \text{Substituted Aldehydes, EtOH, MW, 320 W} \]
\[ \text{d} = \text{Chloroacetyl chloride, triethylamine, DMF, 320 W} \]
3-methyl-1-(2,4-dinitrophenyl)-1H-pyrazol-5(4H)-one (1)

IR (ATR) cm⁻¹: 3356 (NH), 2903 (CH₃ stretch), 1730 (C=O ketone), 1536, 1232 (NO₂), 1367 (CH₃ bend), 1314 (C-N); ¹H NMR (400 MHz, CDCl₃, δ ppm): 2.22 (s, 3H, CH₃), 7.65 (d, 2H, ArH), 7.80 (s, 2H, ArH), 7.95 (s, 1H, NH); mass: m/z 264 (M⁺), 265 (M+1, 12.7%); 266 (M+2, 1.11).

Procedure for the preparation of ethyl 2-(4,5-dihydro-3-methyl-1-(2,4-dinitrophenyl)-5-oxo-1H-pyrazol-4-yl)acetate (2)

Mix Compound 1 and chloroethyl acetate (0.01 mole) in 1,4-dioxane (15 ml) was refluxed for 8 hours. The reaction mixture further stirred for 1 hour and poured in water. The resulting mixture was filtered and recrystallised from ethanol.

Ethyl 2-(4,5-dihydro-3-methyl-1-(2,4-dinitrophenyl)-5-oxo-1H-pyrazol-4-yl)acetate (2)

IR (KBr, cm⁻¹): 3371, 3360 (NH₂), 3328 (NH), 2895 (CH₃ stretch), 1728 (C=O ester), 1710 (C=O ketone), 1513 & 1340 (NO₂), 1321 (CH₃ bend), 1289 (C-N); ¹H NMR (400 MHz, CDCl₃, δ ppm): 1.15 (t, 3H, CH₃), 2.22 (s, 3H, CH₃), 7.65 (d, 2H, ArH), 7.80 (s, 2H, ArH), 7.95 (s, 1H, NH); mass: m/z 350 (M⁺), 351 (M+1, 15.80%).

Procedure for the preparation of 2-(4,5-dihydro-3-methyl-1-(2,4-dinitrophenyl)-5-oxo-1H-pyrazol-4-yl)acetohydrazide (3)

Compound 2 (0.01 mole) and hydrazine hydrate (0.02 mole) were refluxed in absolute methanol (15 ml) for 20 hours. The mixture was concentrated, cooled and poured in ice cold water. The solid thus separated out was filtered, dried and recrystallized from ethanol.

2-(4,5-dihydro-3-methyl-1-(2,4-dinitrophenyl)-5-oxo-1H-pyrazol-4-yl)acetohydrazide (3)

IR (KBr, cm⁻¹): 3344 (NH), 2986 (CH₃ stretch), 1740 (C=O ester), 1710 (C=O ketone), 1513 & 1340 (NO₂), 1321 (CH₃ bend), 1289 (C-N); ¹H NMR (400 MHz, CDCl₃, δ ppm): 1.70 (q, 2H, CH₂), 1.50 (t, 3H, CH₃), 2.22 (s, 3H, CH₃), 7.65 (d, 2H, ArH), 7.80 (s, 2H, ArH), 7.95 (s, 1H, NH); mass: m/z 350 (M⁺), 351 (M+1, 15.80%).

N-(p-hydroxy benzylidenyl)-[2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)acetohydrazide] (4a)

IR (KBr, cm⁻¹): 3350 (NH), 2881 (CH₃ stretch), 1724 (C=O ketone), 1650 (C=N), 1372 (CH₃ bend), 1540 and 1330 (NO₂), 1293 (C-N); ¹H NMR (400 MHz, CDCl₃, δ ppm): 1.50 (s, 2H, CH₂), 2.25 (s, 3H, CH₃), 3.45 (s, 1H, NH), 4.10 (s, 1H, OH), 7.15 (d, 1H, ArH), 7.40 (d, 1H, ArH), 7.55 (m, 4H, ArH), 8.10 (s, 1H, NH); mass: m/z 467 (M⁺), 468 (M+1, 23.10%).

N-(p-nitro benzylidenyl)-[2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)acetohydrazide] (4b)

IR (KBr, cm⁻¹): 3450 (OH), 3350 (NH), 2881 (CH₃ stretch), 1724 (C=O ketone), 1650 (C=N), 1372 (CH₃ bend), 1540 and 1330 (NO₂), 1293 (C-N); ¹H NMR (400 MHz, CDCl₃, δ ppm): 1.70 (q, 2H, CH₂), 1.50 (t, 3H, CH₃), 2.22 (s, 3H, CH₃), 7.65 (d, 1H, ArH), 7.80 (d, 1H, ArH), 7.95 (s, 1H, NH); mass: m/z 440 (M⁺), 441 (M+1, 24.50%).

N-(p-chloro benzylidenyl)-[2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)acetohydrazide] (4c)

IR (KBr, cm⁻¹): 3350 (NH), 2881 (CH₃ stretch), 1724 (C=O ketone), 1650 (C=N), 1372 (CH₃ bend), 1540 and 1330 (NO₂), 1293 (C-N); ¹H NMR (400 MHz, CDCl₃, δ ppm): 1.95 (s, 2H, CH₂), 2.20 (s, 3H, CH₃), 3.40 (s, 1H, NH), 7.10 (d, 1H, ArH), 7.30 (d, 1H, ArH), 7.50 (m, 4H, ArH), 8.00 (s, 1H, NH); mass: m/z 469 (M⁺), 470 (M+1, 24.10%).

N-(p-chloro benzylidenyl)-[2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)acetohydrazide] (4d)

IR (KBr, cm⁻¹): 3355 (NH), 2880 (CH₃ stretch), 1728 (C=O ketone), 1655 (C=N), 1370 (CH₃ bend), 1540 and 1330 (NO₂), 1290 (C-N), 610 (Cl); ¹H NMR (400 MHz, CDCl₃, δ ppm): 1.90 (s, 2H, CH₂), 2.30 (s, 3H, CH₃), 3.45 (s, 1H, NH), 7.00 (d, 1H, ArH), 7.35 (d, 1H, ArH), 7.55 (m, 4H, ArH), 8.10 (s, 1H, NH); mass: m/z 458 (M⁺), 459 (M+2, 21.45%).

N-(p-methyl benzylidenyl)-[2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)acetohydrazide] (4e)

IR (KBr, cm⁻¹): 3355 (NH), 2885 (CH₃ stretch), 1728 (C=O ketone), 1655 (C=N), 1375 (CH₃ bend), 1540 and 1330 (NO₂), 1290 (C-N); ¹H NMR (400 MHz, CDCl₃, δ ppm): 1.95 (s, 2H, CH₂), 2.10 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 3.40 (s, 1H, NH), 7.00 (d, 1H, ArH), 7.35 (d, 1H, ArH), 7.55 (m, 4H, ArH), 8.10 (s, 1H, NH); mass: m/z 438 (M⁺), 439 (M+2, 24.20%).
Procedure for the preparation of 2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)-acetamide-(3-chloro-4-substituted phenylazetidin-2-one) (RSa-RSe)

The Schiff bases (4a-4d) (0.0017 mol) in DMF was taken in a round bottom flask. To it chloroacetyl chloride (0.2 ml) and triethylamine (0.2 ml) were added slowly. Then it was irradiated in a microwave oven (300 W) for appropriate time. Reaction mixture diluted with ice-cold water. The solid product formed was filtered, dried and recrystallised from ethanol.

2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)-acetamide-(3-chloro-4-(p-N,N-dimethylamino)-phenylazetidin-2-one) (RSa)

IR (KBr, cm⁻¹): 3350 (NH), 2885 (CH₃ stretch), 1728 (C=O ketone), 1685 (C=O), 1372 (CH₃ bend), 1540 and 1330 (NO₂), 1293 (C-N), 610 (Cl); ¹H NMR (400 MHz, CDCl₃, δ ppm), 1.50 (s, 2H, CH₂), 2.25 (s, 3H, CH₃), 3.45 (s, 1H, NH), 4.10 (s, 1H, OH), 5.15 (d, 1H, CH), 5.35 (d, 1H, CH), 7.15 (d, 1H, ArH), 7.40 (d, 1H, ArH), 7.55 (m, 4H, ArH), 8.10 (s, 1H, NH); mass: m/z 543 (M⁺), 544 (M⁺+1, 32.10%).

2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)-acetamide-(3-chloro-4-(p-hydroxy)-phenylazetidin-2-one) (RSb)

IR (KBr, cm⁻¹): 3450 (OH), 3350 (NH), 2881 (CH₃ stretch), 1724 (C=O ketone), 1685 (C=O), 1372 (CH₃ bend), 1540 and 1330 (NO₂), 1293 (C-N), 610 (Cl); ¹H NMR (400 MHz, CDCl₃, δ ppm), 1.50 (s, 2H, CH₂), 2.25 (s, 3H, CH₃), 3.45 (s, 1H, NH), 4.10 (s, 1H, OH), 5.15 (d, 1H, CH), 5.35 (d, 1H, CH), 7.15 (d, 1H, ArH), 7.40 (d, 1H, ArH), 7.55 (m, 4H, ArH), 8.10 (s, 1H, NH); mass: m/z 516 (M⁺), 517 (M⁺+1, 25.10%).

2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)-acetamide-(3-chloro-4-(p-nitro)-phenylazetidin-2-one) (RSc)

IR (KBr, cm⁻¹): 3350 (NH), 2881 (CH₃ stretch), 1724 (C=O ketone), 1685 (C=O), 1372 (CH₃ bend), 1540 and 1330 (NO₂), 1293 (C-N), 610 (Cl); ¹H NMR (400 MHz, CDCl₃, δ ppm), 1.50 (s, 2H, CH₂), 2.25 (s, 3H, CH₃), 3.45 (s, 1H, NH), 4.10 (s, 1H, OH), 5.15 (d, 1H, CH), 5.35 (d, 1H, CH), 7.15 (d, 1H, ArH), 7.40 (d, 1H, ArH), 7.55 (m, 4H, ArH), 8.10 (s, 1H, NH); mass: m/z 516 (M⁺), 517 (M⁺+1, 25.10%).

2-(4,5-dihydro-3-methyl-5-oxo-1-(2,4-dinitrophenyl)-1H-pyrazol-4-yl)-acetamide-(3-chloro-4-(p-chloro)-phenylazetidin-2-one) (RSe)

IR (KBr, cm⁻¹): 3355 (NH), 2885 (CH₃ stretch), 1728 (C=O ketone), 1685 (C=O), 1375 (CH₃ bend), 1540 and 1330 (NO₂), 1290 (C-N), 610 (Cl); ¹H NMR (400 MHz, CDCl₃, δ ppm), 1.95 (s, 2H, CH₂), 2.10 (s, 3H, CH₃), 2.35 (s, 3H, CH₃), 3.40 (s, 1H, NH), 5.25 (d, 1H, CH), 5.40 (d, 1H, CH), 7.00 (d, 1H, ArH), 7.35 (d, 1H, ArH), 7.55 (m, 4H, ArH), 8.10 (s, 1H, NH); mass: m/z 514 (M⁺), 515 (M⁺+1, 26.30%).

Antibacterial Activity

Applying cup plate technique all of the newly synthesized compounds were screened in vitro for antibacterial activity against Escherichia coli (Gram-negative), Bacillus subtilis (Gram-positive) at 10 μg/ml, 20 μg/ml, 30 μg/ml concentrations, respectively. Under identical conditions, the antibiotics ampicillin at 10 μg/ml, 20 μg/ml, 30 μg/ml exhibited 14, 16, 18 mm zone of inhibition for Gram negative organism and exhibited 13, 15, 19 mm zone of inhibition for Gram-positive organism respectively.

Conclusion

Various 2-(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)-acetamide-(3-chloro-4-substituted phenylazetidin-2-one) (RSa-RSe) derivatives were synthesized and screened for antibacterial activity. Compounds (4a-4e) reacted with chloroacetyl chloride and triethylamine in microwave oven to give corresponding phenylazetidin-2-one (RSa-RSe) in very good yields. It was interesting to note that out of five compounds RSa, Rsb, RSe were found to have antibacterial activity near to standard. In view of these observations, we conclude that (RSa-RSe) could be developed as a novel class of antibacterial agents. However, further in detailed antibacterial screening with QSAR studies is required to identify the potent molecule without severe side effects.

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References


