Synthesis of new pyrazolines and their biological evaluation as antimicrobial agents

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2-Diazopropane and diazo compounds derived from aromatic aldehydes were reacted with aromatic esters of 3-hydroxyprop-1-yne to give the aromatic esters of 5-hydroxymethyl-3-substituted pyrazoles. Anti-microbial screening using the Gram positive (*Staphylococcus aureus* and *Enterococcus fecalis*) and Gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria and the yeast *Candida albicans*, showed that these compounds had promising activity against both Gram positive and Gram negative bacteria.

Keywords: diazo compounds, alkynes, cycloaddition 1,3-dipolar, pyrazoles, antimicrobial properties

We have been interested in the development of novel ring systems because of their potential ability to influence the pharmacokinetic properties of compounds in beneficial ways for the drug discovery process.[1,4] The pyrazole motif makes up the core structure of numerous biologically active compounds. Thus, some representatives of this heterocycle exhibit antiviral/antitumour,[5,6] antibacterial,[7] antiinflamatory,[8] analgesic,[9] fungistatic,[10] NO-donor antioxidants[11] and XO inhibitory activity.[12] In recent years, there has been a need for rapid reactions that meet the three main criteria of an ideal synthesis: efficiency, versatility, and selectivity. Such reactions would allow medicinal chemistry to keep pace with the information derived from modern biological screening techniques.[13] In connection with our medicinal chemistry project aimed at the discovery of new antibacterial agents, we needed to prepare a series of pyrazoles. A valid synthesis is represented by the 1,3-dipolar cycloaddition of diazoalkanes with alkynes.[14] The advantage of this procedure is that it is often highly regioselective and, in addition, the 1,3-dipole can react with an array of unsaturated substrates to produce a number of different heterocyclic entities.

We report here the synthesis of some new pyrazole derivatives which have been found to possess an interesting profile of antibacterial activity.

Results and discussion

The acetylenes **1a–d** reacted with 2-diazopropane **2**,[15] to afford the regioisomers **3a–d** where the diazo carbon atom attacks the terminal acetylenic carbon and the terminal diazo nitrogen atom bonds to the central carbon atom of the acetylene (Scheme 1).[16]

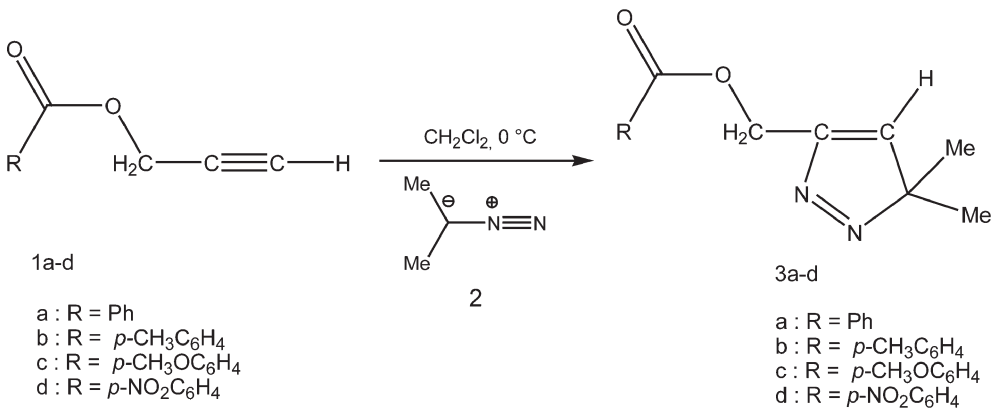
Other diazo compounds that have been investigated include the 1-aryldiazomethanes **5a–c**. Condensation of *p*-toluenesulfonyl hydrazide with an aromatic aldehyde followed by treatment with an aqueous solution of sodium hydroxide led to a solution of aromatic aldehyde tosylhydrazone sodium salt, which upon warming to 50 °C gave a reddish solution of diazo compounds.[17] Prior to warming the reaction mixture, the acetylenes **1b–d** were added and the desired pyrazoles **6a–f** were obtained in good yield as single regioisomers (Scheme 2).

Antimicrobial activity

The antimicrobial activity of compounds **3** and **6** was determined by the agar dilution technique as recommended by the Clinical and Laboratory Standard Institute (CLSI) [18] utilising a variety of Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus fecalis*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and the yeast (*Candida albicans*). From the results which were obtained (Table 1) it has been noticed that all the tested compounds exhibit promising antimicrobial properties against both Gram-positive and Gram-negative bacteria. However, all the tested compounds seem completely inactive against the yeast (*C. albicans*) which was used. It is also noted that, in general, compounds **6** are more effective antibacterial agents than compounds **3**. Compounds **6c** and **6f** seem to be the most effectively prepared Gram-negative antibacterial agents. This may be attributed to the role of the anisyl function attached to the 3-position of the pyrazole ring system as it enhances antibacterial properties to the greatest extent compared to the other residues.

Conclusions

In conclusion, our studies have revealed that the 1,3-dipolar cycloaddition of diazo compounds with various alkynes gives



Scheme 1 Formation of pyrazoles using 2-diazopropane.

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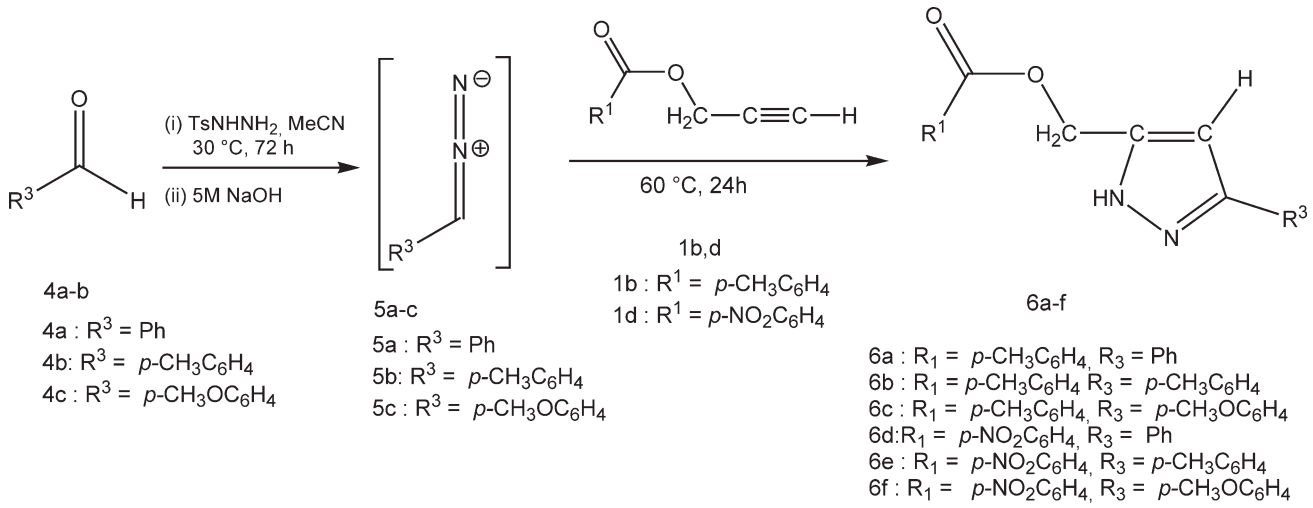

Scheme 2 Proposed synthesis of pyrazoles using diazo compounds.

pyrazoles with complete regioselectivity. These compounds were tested for antimicrobial properties against both Gram-positive and Gram-negative bacteria. From these studies compounds **6c** and **6f** have emerged as the lead compound, showing maximum antimicrobial activity. Thus, compound **6c** represents a fruitful matrix for development of a new class of antimicrobial agent that deserve further investigation and derivatisation.

Experimental

All solvents were distilled prior to use. Chromatography was performed with silica gel 60 (230–400 mesh), and silica gel F254 plates were used for preparative TLC. The IR spectra frequencies are gives in cm^{-1}. NMR spectra were determined in $CDCl_3$ solutions at 300 and 75.5 MHz for ^1H and ^{13}C NMR, respectively; chemical shifts (δ) were reported in ppm and J values are given in Hz. Microanalyses were performed using a Carlo Erba EA1108. All preparations involving diazopropane were carried out in an efficient fume cupboard behind a safety screen.

Synthesis of the 1,3-dipolar cycloaddition of 2-diazopropane with alkynes **1a–d**

A 2.6 M ethereal solution of 2-diazopropane was added portionwise to a solution of alkynes **1a–d** (1.5 mmol) in diethyl ether, cooled at –10 °C. The reaction was kept at the same temperature during 1h. The solvent was removed and chromatography (SiO_2; ethyl acetate/petroleum ether, 1:3) afforded compounds **3a–d**.

(3,3-Dimethyl-3H-pyrazol-5-yl)methyl benzoate (**3a**): Yield, 70%; m.p. 133 °C. Anal. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.83; H, 6.17; N, 12.20%; IR (KBr) ν_{cm}^{-1}; 1540 (N=N); 1745 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ: 1.46 (s, 6H, CH_3), 5.49 (s, 2H, CH_2), 6.98 (s, 1H, CH), 7.34–7.55 (m, 5H, H_{arom}); $^{13}C\{^1H\}$NMR (75 MHz, $CDCl_3$) δ: 20.19 (CH_3), 21.65, 59.11 (CH_2) 93.39 (C-5), 128.41–133.09 (C_{arom}), 144.66 (C-3), 152.00 (C-4), 167.24 (C=O).

(3,3-Dimethyl-3H-pyrazol-5-yl)methyl 4-methylbenzoate (**3b**): Yield, 80%; m.p. 151 °C. Anal. Calcd for $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.76; N, 15.27. Found: C, 57.01; H, 4.68; N, 15.20%; IR (KBr) ν_{cm}^{-1}; 1545 (N=N); 1740 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ: 1.47 (s, 6H, CH_3), 5.57 (s, 2H, CH_2), 6.96 (s, 1H, CH), 8.24 (d, 2H, H_{arom}, J = 9 Hz); 8.33 (d, 2H, H_{arom}, J = 9 Hz); $^{13}C\{^1H\}$NMR (75 MHz, $CDCl_3$) δ: 20.21 (CH_3), 21.63 (CH_3), 59.09 (CH_2) 93.36 (C-5), 123.45–152.05 (C_{arom}), 144.81 (C-3), 151.33 (C-4), 167.21 (C=O).

(3,3-Dimethyl-3H-pyrazol-5-yl)methyl 4-methoxybenzoate (**3c**): Yield, 75%; m.p. 157 °C. Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.76; H, 6.55; N, 11.59%; IR (KBr) ν_{cm}^{-1}; 1540 (N=N); 1735 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ: 1.44 (s, 6H, CH_3), 2.41 (s, 3H, CH_3), 5.51 (s, 2H, CH_2), 6.86 (s, 1H, CH), 7.23 (d, 2H, H_{arom}, J = 7.8 Hz); 7.95 (d, 2H, H_{arom}, J = 7.8 Hz); $^{13}C\{^1H\}$NMR (75 MHz, $CDCl_3$) δ: 20.24 (CH_3), 21.69 (CH_3), 59.17 (CH_2) 94.16 (C-5), 126.95–144.05 (C_{arom}), 143.92 (C-3), 151.19 (C-4), 166.20 (C=O).

(3,3-Dimethyl-3H-pyrazol-5-yl)methyl 4-nitrobenzoate (**3d**): Yield, 85%; m.p. 148 °C. Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.58; H, 6.15; N, 10.87%; IR (KBr) ν_{cm}^{-1}; 1545 (N=N); 1740 (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ: 1.45 (s, 6H, CH_3), 3.86 (s, 3H, OCH_3), 5.49 (s, 2H, CH_2), 6.86 (s, 1H, CH), 6.87 (d, 2H, H_{arom}, J = 9 Hz); 8.02 (d, 2H, H_{arom}, J = 9 Hz); $^{13}C\{^1H\}$NMR (75 MHz, $CDCl_3$) δ: 20.25 (CH_3), 55.47 (OCH_3), 59.07 (CH_2) 94.16 (C-5), 113.74–163.68 (C_{arom}), 143.90 (C-3), 151.29 (C-4), 165.88 (C=O).

Synthesis of pyrazoles **6a–f***; general procedure*

The aromatic aldehydes were added to a solution of *p*-toluenesulfonylhydrazide (1.5 mmol). After stirring for 3 h at room temperature, a solution of 5N NaOH (300 μL, 1.5 mmol) was added and the mixture was stirred for a further 20 min. The alkynes **1b** or **1d** (7.5 mmol) were added and the mixture was stirred at 50 ºC for 48 h. The volatiles were evaporated under reduced pressure and the residue was dissolved in a 1:1 mixture of water–ethyl acetate (70 mL). The organic layer was separated and dried over $MgSO_4$. After filtration and removal of the solvent under reduced pressure, the crude material was purified by flash chromatography (eluent petroleum ether/ethyl acetate 4:1) to afford a white solid.

(3-Phenyl-1H-pyrazol-5-yl)methyl 4-methylbenzoate (**6a**): Yield, 60%; m.p. 161 °C. Anal. Calcd for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N,

Table 1 Minimum inhibitory concentrations (MIC, mg mL^{-1}) of the tested compounds against different organisms

Compounds	**3a**	**3b**	**3c**	**3d**	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	CIP	AMP
Organisms												
Staphylococcus aureus	10	10	10	10	5	0.6	2.5	10	2.5	5	0.3	(–)
Enterococcus fecalis	2.5	10	10	5	2.5	1.3	5	0.6	1.3	2.5	0.3	(–)
Escherichia coli	10	10	5	10	2.5	2.5	0.6	2.5	1.3	0.6	0.3	(–)
Klebsiella pneumoniae	5	5	10	10	2.5	5	1.3	5	1.3	2.5	1.3	(–)
Candida albicans	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(–)	4

CIP, Ciprofloxacin; AMP, Amphotericin B; (–), inactive.

9.58. Found: C, 73.91; H, 5.55; N, 9.59%; IR (KBr) ν_{cm}^{-1}; 1635 (C=N); 1740 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.39 (s, 3H, CH_3), 5.49 (s, 2H, CH_2), 6.83 (s, 1H, CH), 6.82–7.45 (m, 5H, H_{arom}); 7.63 (d, 2H, H_{arom}, J = 8.1 Hz); 7.76 (d, 2H, H_{arom}, J = 8.1 Hz); ^{13}C{^{1}H}NMR (75 MHz, $CDCl_3$) δ: 21.31 (CH_3), 59.21 (CH_2), 101.10 (C-4), 125.47–142.07 (C_{arom}), 148.16 (C-5), 153.02 (C-3), 167.21 (C=O).

(3-p-Tolyl-1H-pyrazol-5-yl)methyl 4-methylbenzoate (**6b**): Yield, 70%; m.p. 173 °C. Anal. Calcd for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14. Found: C, 74.51; H, 5.96; N, 9.17%; IR (KBr) ν_{cm}^{-1}; 1630 (C=N); 1740 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.41 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 5.52 (s, 2H, CH_2), 6.67 (s, 1H, CH), 7.23–7.98 (m, 8H, H_{arom}); ^{13}C{^{1}H}NMR (75 MHz, $CDCl_3$) δ: 21.23 (CH_3), 21.53 (CH_3), 59.21 (CH_2), 101.21 (C-4), 126.32–144.17 (C_{arom}), 148.23 (C-5), 152.98 (C-3), 168.22 (C=O).

(3-(4-Methoxyphenyl)-1H-pyrazol-5-yl)methyl 4-methylbenzoate (**6c**): Yield, 80%; m.p. 161 °C. Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.73; H, 5.66; N, 8.72%; IR (KBr) ν_{cm}^{-1}; 1645 (C=N); 1745 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.41 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 5.56 (s, 2H, CH_2), 6.69 (s, 1H, CH), 6.85 (d, 2H, H_{arom}, J = 9 Hz); 8.11 (d, 2H, H_{arom}, J = 9 Hz); 7.64 (d, 2H, H_{arom}, J = 8.1 Hz); 7.71 (d, 2H, H_{arom}, J = 8.1 Hz); ^{13}C{^{1}H}NMR (75 MHz, $CDCl_3$) δ: 21.19 (CH_3), 55.81 (OCH_3), 59.31 (CH_2), 102.01 (C-4), 121.33–161.16 (C_{arom}), 149.18 (C-5), 153.04 (C-3), 169.01 (C=O).

(3-Phenyl-1H-pyrazol-5-yl)methyl 4-nitrobenzoate (**6d**): Yield, 55%; m.p. 147 °C. Anal. Calcd for $C_{17}H_{13}N_3O_4$: C, 63.16; H, 4.05; N, 13.00. Found: C, 63.23; H, 4.09; N, 13.03%; IR (KBr) ν_{cm}^{-1}; 1640 (C=N); 1745 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.46 (s, 2H, CH_2), 6.79 (s, 1H, CH), 6.83–7.44 (m, 5H, H_{arom}); 8.25 (d, 2H, H_{arom}, J = 9 Hz); 8.33 (d, 2H, H_{arom}, J = 9 Hz); ^{13}C{^{1}H}NMR (75 MHz, $CDCl_3$) δ: 59.26 (CH_2), 102.25 (C-4), 124.49–151.09 (C_{arom}), 149.09 (C-5), 152.97 (C-3), 166.09 (C=O).

(3-p-Tolyl-1H-pyrazol-5-yl)methyl 4-nitrobenzoate (**6e**): Yield, 75%; m.p. 135 °C. Anal. Calcd for $C_{18}H_{15}N_3O_4$: C, 64.09; H, 4.48; N, 12.46. Found: C, 64.11; H, 4.52; N, 12.50%; IR (KBr) ν_{cm}^{-1}; 1640 (C=N); 1745 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.41 (s, 3H, CH_3), 5.47 (s, 2H, CH_2), 6.69 (s, 1H, CH), 7.24 (d, 2H, H_{arom}, J = 7.8 Hz); 7.91 (d, 2H, H_{arom}, J = 7.8 Hz); 8.21 (d, 2H, H_{arom}, J = 9 Hz); 8.29 (d, 2H, H_{arom}, J = 9 Hz); ^{13}C{^{1}H}NMR (75 MHz, $CDCl_3$) δ: 21.17 (CH_3), 59.23 (CH_2), 102.09 (C-4), 119.44–152.17 (C_{arom}), 149.10 (C-5), 152.99 (C-3), 166.22 (C=O).

(3-(4-Methoxyphenyl)-1H-pyrazol-5-yl)methyl 4-nitrobenzoate (**6f**): Yield, 80%; m.p.139 °C. Anal. Calcd for $C_{18}H_{15}N_3O_5$: C, 61.19; H, 4.28; N, 11.89. Found: C, 61.21; H, 4.22; N, 11.90%; IR (KBr) ν_{cm}^{-1}; 1640 (C=N); 1745 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.39 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 6.61 (s, 1H, CH), 6.88 (d, 2H, H_{arom}, J = 9 Hz); 8.01 (d, 2H, H_{arom}, J = 9 Hz); 8.23 (d, 2H, H_{arom}, J = 9 Hz); 8.28 (d, 2H, H_{arom}, J = 9 Hz);^{13}C{^{1}H}NMR (75 MHz, $CDCl_3$) δ: 55.79 (OCH_3), 59.24 (CH_2), 102.21 (C-4), 123.08–160.11 (C_{arom}), 149.15 (C-5), 153.01 (C-3), 166.29 (C=O).

Antimicrobial activity

The antimicrobial activity of the synthesised compounds **3** and **6** was determined by the agar dilution technique as recommended by the Clinical and Laboratory Standard Institute (CLSI).[18] The tested compounds were dissolved in dimethyl sulfoxide (DMSO). An inoculum of about 1.5–108 colony forming unit per spot was applied to the surfaces of Mueller–Hinton agar plates containing graded concentrations of the respective compound; plates were incubated at 37 °C for 18 h. The spot with the lowest concentration of compound showing no growth was defined as the minimum inhibitory concentration (MIC). All organisms used in this study were standard strains obtained from American Type Culture Collection. The organisms included representatives of Gram-positive bacteria (*S. aureus* 25923 and *E. fecalis* 29212), Gram-negative bacteria (*E. coli* 25922 and *K. pneumoniae* 33495) and yeast (*C. albicans* 20260). The MIC of ciprofloxacin and amphotericin B was determined concurrently as reference for antibacterial activities, respectively (Table 1). Control DMSO was carried out with each experiment.

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