Synthesis and antimicrobial activity of pyrazole derivatives via 1,3-dipolar cycloaddition of nitrile imines with ethyl acetoacetate

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The ethyl acetoacetate reacts with the nitrile imines generated in situ by the catalytic dehydrogenation of diphenyl hydrazones using chloramine-T (CAT) to afford regioselective cycloadducts in 80% yields respectively. The structures of these compounds have been characterized by FT-IR, ¹H NMR, ¹³C NMR and mass spectroscopic techniques and elemental analysis. All the pyrazole derivatives have been tested for their antibacterial and antifungal activities.

Key words: 1,3-dipolar cycloaddition, nitrile imines, pyrazoles.

INTRODUCTION

Heterocyclic compounds are considered as the most promising molecules for the design of new drugs. 1,3-Dipolar cycloaddition reactions are an efficient synthetic tool for constructing biologically potent five membered heterocyclic compounds [1, 2]. Pyrazoles, pyrazolines, pyrazolidines and pyrazolones are gaining importances as biologically active compounds possessing such as analgesic, antipyretic, antiinflammatory, germicidal and anti-fungal activities [3, 4], antiprotozoal [5], fungicidal [6], bactericidal [6], herbicidal and plant growth regulating properties.

Apart from the various dipolar reagent nitrile imines are used in numerous 1,3-dipolar cycloaddition reaction leading to pyrazoles, pyrazolines, pyrazolidines and other heterocyclic compounds [7]. Huisgen and co-workers first reported [8] the authentic in situ generation of nitrile imines by the thermolysis of 2,5-diphenyl tetrazole in the presence of ethyl phenyl propiolate and obtained 2,3,5-triphenyl carbethoxypyrazole. The usual synthesis of nitrile imines involves the thermolysis or photolysis of tetrazole [8], oxidation of aldehyde hydrazones with lead tetra acetate [9], CAT [10] and mercuric acetate [11].

In addition to this, nitrile imines are known to react with heterocyclic compounds to yield a variety of polyheterocycles [12]. Shawali and co-workers [13] prepared a numerous pyrazole derivatives by the reaction of in situ generated nitrile imines obtained from hydrazidoyl halides with sodium salt of active methylene compounds, such as β-keto-sulphones, β-ketoamides and β-cyanoketones. Baruah et al. [14] generated the C-acetyl and C-ethoxy carbonyl nitrile imines in situ from the corresponding hydrazonoyl halides in the presence of triethylamine in anhydrous chloroform, and have used these nitrile imines for the preparation of pyrazoles derivatives. The intramolecular cycloaddition of in situ generated nitrile imine with aldonitrones afforded triazoles [15]. Mogilaiah et al. [16] developed a solvent free method for the facile synthesis of 1,8-naphthyridinyl-pyrazoles using POCl₃-DMF (Vilsmeier-Haack reagent) over silica gel under microwave irradiation. Aly et al. [17] showed a new synthetic route for the synthesis of some pyrazole derivatives from 3-aryl-1-phenyl-1H-pyrazole-4-carbaldehydes.

Padmavathi and co-workers [18] prepared activated bis pyrazolines and bis isoxazolines by 1,3-dipolar cycloaddition of nitrile imines and nitrile oxides to activated bis olefinic systems in the presence of Chloramine-T. Bacchetti [19] prepared 1,4-dicarboxethoxy pyrazoles by intermolecular cycloaddition of nitrile imines with ethyl acetoacetate. Though there are more references available in the literature on cycloaddition of nitrile imines with alkenes and alkynes, there is a less information about the use of keto-enol tautomers as dienophile for the cycloaddition. We have synthesized [20] the 1-(5-methyl-1,3-diphenyl-1H-pyrazol-4-yl)-ethanone in quantitative yield via 1,3-dipolar cycloaddition of enol form of acetyl acetone with the nitrile imines generated in situ by the catalytic dehydrogenation of diphenyl hydrazones using CAT. This prompted us to work in this area in detail to make it as a general method for the synthesis of pyrazoles derivatives.

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RESULTS AND DISCUSSION

It is well known that acetyl acetone and ethyl acetoacetate exist in two dynamic equilibrium states via, keto and enol forms. It is also known that typical cycloaddition of nitrile imines with alkenes and alkynes afford pyrazolines and pyrazoles respectively. It is interesting to note that, though the expected products are pyrazolines as similar to that of addition of nitrile imines to alkenes, the reaction afforded pyrazoles with loss of water molecule (Scheme).

In typical reaction, a mixture of aldehyde hydrazone 1a with excess of ethyl acetoacetate 3 and CAT in glacial acetic acid was stirred at room temperature for about 2–3 hours. After the usual work up, 5a was isolated as light yellow oil in 80% yield. In similar manner, 1b–g were converted into the corresponding pyrazole derivatives 5b–g in good yields. IR, 1H NMR, 13C NMR, MS studies and elemental analysis provide the structural proof for the products. In 1H NMR spectra, the signals for the ethoxy protons appears as a quartet in the region δ 4.12–4.31 ppm, (2H, J = 7.2 Hz, –OCH₂–CH₃), while the protons for the methyl group at C-5 appear as a singlet in the region δ 2.68–2.75 ppm. The proton signals for the methyl group at C-5 is probably due to deshielding by the –CO–OC₂H₅ group. These observations clearly indicate that the formation of the cycloadduct 5a is obtained via pyrazolines 4 with the loss of water molecule. In 13C NMR spectra, the –C-3 and C-4 appear as singlet δ 176.14–176.26 ppm. All compounds were converted into the corresponding pyrazolines as similar to that of addition of nitrile imines to alkenes, the reaction afforded pyrazoles with loss of water molecule (Scheme).

ANTIMICROBIAL SCREENING

Synthesized pyrazoles (5a–g) were screened (dose of 100 µg) for their antibacterial activity against Gram-negative bacteria Escherichia coli (E. coli) and Gram-positive bacteria Staphylococcus aureus (S. aureus) using filter paper disc method [21]. Plates inoculated with E. coli were incubated for 48 h and plates inoculated with S. aureus for 24 h respectively at room temperature. Streptomycin sulphate was used as a standard. After the period of incubation the inhibition zones were measured in mm and results obtained are shown in Table 1. All the compounds were also screened (dose of 100 µg) for their antifungal activity against Candida albicans (C. albicans) and Aspergillus niger (A. niger) using Griseofulvin as a standard. The results are shown in Table 1. Compared with Streptomycin sulphate the compounds 5b and 5e–g showed moderate antibacterial activity against E. coli and 5b and 5f against S. aureus. Compared with the standard Griseofulvin the compounds 5b–c, 5e and 5f showed promising antifungal activity against C. albicans and 5f against A. niger.

Table 1. Antibacterial and antifungal activity of synthesized pyrazole derivatives (5a–g). (Zone of inhibition in mm).

<table>
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<tr>
<th>Compounds</th>
<th>Antibacterial activity</th>
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Griseofulvin: Not tested Not tested 14 12
EXPERIMENTAL SECTION

The purity of all synthesized compounds was checked by thin layer chromatography using silica gel G. The final compounds were purified by column chromatography on silica gel (70–230 mesh, Merck) using mixture of chloroform:acetone (7:1) as eluent. 1H NMR spectra were registered either on a Bruker 300 MHz or Jeol 60 MHz Hitachi Perkin Elmer spectrometer, and 13C NMR spectra on a Jeol GSX 400 (75 MHz) instrument using 1% tetramethylsilane as internal standard (chemical shifts are expressed in δ, ppm downfield from the tetramethylsilane). Mass spectra were obtained on an electron impact Masspec MSW 9629 spectrometer and important fragments are given with the relative intensities in brackets.

Typical procedure for the preparation of ethyl 5-methyl-1,3-diphenyl-1H-pyrazole-4-carboxylate (5a): A mixture of benzaldehyde hydrazone (1a, 2.35 g, 12.0 mmol), excess of freshly distilled ethyl acetoacetate (2.34 g, 18.0 mmol) as an oily substance in 78% yield (3.14 g). The pyrazole substance in 80% yield showed IR bands (Nujol) ν: 1716 cm–1 (C=O), 1618 cm–1 (C=N), 1596 cm–1 (C=C); 1H NMR (CDCl3): δ 1.02 (q, 1C, H 3C–C(5)), 2.70 (s, 3C–C(5)), 3.75 (s, 5H, –OCH3), 55.80 (q, 1C, –CH 2–C), 7.05–7.26 (s, 5H, Ar'–H), 7.65–7.78 (m, 5H, Ar'–H); 13C NMR (CDCl3) δ: 124.42 (d, 1C), 124.56 (d, 2C), 126.22 (d, 2C), 128.74 (d, 2C), 130.28 (s, 1C), 131.08 (d, 1C), 132.42 (s, 1C), 161.12 (s, 1C), 178.48* (s, 1C, CO). MS (relative intensity) m/e for C20H19N2O3: 337 (M+1, 100), 307 (32), 263 (40), 248 (20), 224 (24), 112 (16), 133 (78), 91 (46), 88 (10), 29 (24). Anal. Calcd: C, 71.41; H, 5.99; N, 8.33%. Found: C, 71.38; H, 5.87; N: 8.25%.

Ethyl 3-(3,4-dimethoxyphenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylate (5c): Obtained from 3,4-dimethoxybenzaldehyde hydrazone (1c (2.56 g, 10 mmol), ethyl acetoacetate (2.34 g, 18.0 mmol) as an oily substance in 82% yield (2.74 g). IR bands (Nujol) ν: 1720 cm–1 (C=O), 1622 cm–1 (C=N), 1596 cm–1 (C=C); 1H NMR (CDCl3): δ 1.24 (t, 3H, J = 7.0 Hz, –OCH2–C), 2.68 (s, 3H, H–C(5)), 3.75 (s, 6H, –OCH3), 4.16 (q, 2H, J = 7.1 Hz, –OCH2–C), 6.98–7.12 (m, 3H, Ar'–H), 7.48–7.66 (m, 5H, Ar'–H); 13C NMR (CDCl3) δ: 1.02 (q, 1C, H–C–(5)), 136.36 (s, 1C), 131.12 (d, 1C), 132.44 (s, 1C), 160.82 (s, 1C), 176.20* (s, 1C, 5-C), 171.68* (s, 1C, CO). MS (relative intensity) m/e for C24H22N2O6: 377 (M+1, 100), 307(32), 263 (40), 248 (20), 224 (24), 112 (16), 133 (78), 91 (46), 88 (10), 29(24). Anal. Calcd: C, 71.41; H, 5.99; N, 8.33%. Found: C, 71.38; H, 5.87; N: 8.25%.

Ethyl 5-methyl-1-phenyl-3-(3,4,5-trimethoxyphenyl)-1H-pyrazole-4-carboxylate (5d): Obtained from 3,4,5-trimethoxybenzaldehyde hydrazone (1d (2.86 g, 10 mmol), ethyl acetoacetate (2.34 g, 18.0 mmol) as an oily substance in 81% yield (3.20 g). IR bands (Nujol) ν: 1718 cm–1 (C=O), 1620 cm–1 (C=N), 1600 cm–1 (C=C); 1H NMR (CDCl3): δ: 1.26 (t, 3H, J = 7.1 Hz, –OCH2–C), 2.70 (s, 3H, H–C(5)), 3.71 (s, 9H, –OCH3), 4.22 (q, 2H, J = 7.2 Hz, –OCH2–C), 6.96 (m, 2H, Ar'–H), 7.52–7.68 (m, 5H, Ar'–H); 13C NMR (CDCl3) δ: 1.04 (q, 1C, H–C–(5)), 13.62 (q, 1C, –CH2–C), 56.6 (q, 2C, 3.5'–OCH3), 58.4 (q, 1C, 4'–OCH3), 59.22 (t, 1C, –C 3'–OCH3), 59.62 (t, 1C, –C 5'–OCH3), 59.80 (q, 1C, –CH 2–C), 7.22 (d, 2H, Ar'–H), 7.36–7.48 (m, 5H, Ar'–H); 13C NMR (CDCl3) δ: 1.02 (q, 1C, H–C–(5)), 136.36 (s, 1C), 131.12 (d, 1C), 132.44 (s, 1C), 161.14 (s, 1C), 176.24* (s, 1C, 5-C), 171.72* (s, 1C, CO). MS (relative intensity) m/e for C26H26N2O8: 397 (M+1, 100), 327(39), 293 (39), 278 (23), 254 (28), 163 (76), 112 (14), 91 (46), 88 (12), 29(26). Anal. Calcd: C, 78.84; H, 6.05; N, 7.65%. Found: C, 78.77; H, 5.96; N, 7.54%.
Ethyl 3-(4-chlorophenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylate (5e). Obtained from 4-chlorobenzaldehyde hydrazone 1e (2.76 g, 12 mmol), ethyl acetoacetate (2.6 g, 20.0 mmol) as an oily substance in 79% yield (3.21 g). IR bands (Nujol) v: 1724 cm⁻¹ (C=O), 1616 cm⁻¹ (C=N), 1598 cm⁻¹ (C=C). ¹H NMR (CDCl₃) δ: 1.02 (t, 3H, J = 7.2 Hz, –CH₂–CH₃), 2.74 (s, 3H, H₃C–C(5)), 4.22 (q, 2H, J = 7.1 Hz, –OCH₂–CH₃), 6.98 (d, 2H, Ar'–H), 7.18 (d, 2H, Ar'–H), 7.44–7.60 (m, 5H, Ar″–H); ¹³C NMR (CDCl₃) δ: 12.33 (d, 2C), 124.30 (d, 1C), 127.22 (d, 2C), 130.18 (d, 2C), 132.52 (s, 1C), 134.86 (d, 1C), 138.32 (s, 1C), 161.02 (s, 1C), 176.14* (s, 1C, –CH 2–C); ¹H NMR (CDCl₃) δ: 1.04 (q, 1C, H₃C–C(5)), 13.62 (q, 1C, –CH₂–C); ¹³C NMR (CDCl₃) δ: 12.8 (t, 3H, J = 6.9 Hz, –OCH₂–CH₃), 2.68 (s, 3H, H₃C–C(5)), 4.31 (q, 2H, J = 6.8 Hz, –OCH₂–CH₃), 7.08 (d, 2H, Ar¹–H), 7.24 (d, 2H, Ar²–H), 7.48–7.66 (m, 5H, Ar″–H); ¹³C NMR (CDCl₃) δ: 132.16 (d, 2C), 134.30 (d, 1C), 127.44 (d, 2C), 128.56 (d, 2C), 130.04 (d, 2C), 132.46 (d, 2C), 134.78 (d, 1C), 138.14 (s, 1C), 161.84 (s, 1C), 176.14* (s, 1C, 5-C), 169.22* (s, 1C, CO). MS (relative intensity) m/e for C₂₀H₂₃N₃O₅: 137 (76), 112 (16), 91 (42), 88 (12), 29(26). Anal. Calcd: C, 74.98; H, 5.03; N, 8.22%. Found: C, 66.91; H, 4.90; N, 8.16%.

Ethyl 3-(4-N,N-dimethylaminophenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylate (5f). Obtained from 4-N,N-dimethylbenzaldehyde hydrazone 1f (2.86 g, 12 mmol), ethyl acetoacetate (2.6 g, 20.0 mmol) as an oily substance in 78% yield (3.25 g). IR bands (Nujol) v: 1726 cm⁻¹ (C=O), 1624 cm⁻¹ (C=N), 1598 cm⁻¹ (C=C). ¹H NMR (CDCl₃) δ: 0.96 (q, 1C, H₃C–C(5)), 13.58 (q, 1C, –CH₂–C); ¹³C NMR (CDCl₃) δ: 1.02 (t, 3H, J = 7.2 Hz, –CH₂–CH₃), 2.68 (s, 3H, H₃C–C(5)), 4.22 (q, 2H, J = 6.8 Hz, –OCH₂–CH₃), 7.08 (d, 2H, Ar¹–H), 7.24 (d, 2H, Ar²–H), 7.48–7.66 (m, 5H, Ar″–H); ¹³C NMR (CDCl₃) δ: 12.33 (d, 2C), 124.30 (d, 1C), 127.22 (d, 2C), 130.18 (d, 2C), 132.46 (d, 2C), 134.30 (d, 1C), 138.14 (s, 1C), 161.84 (s, 1C), 176.14* (s, 1C, 5-C), 169.22* (s, 1C, CO). MS (relative intensity) m/e for C₂₁H₂₄N₃O₅: 341 (M+1, 100), 311 (30), 267 (41), 252 (18), 228 (34), 137 (76), 112 (16), 91 (42), 88 (12), 29(26). Anal. Calcd: C, 66.65; H, 6.10; N, 7.07%. Found: C, 66.56; H, 5.98; N, 7.04%.

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REFERENCE

СИНТЕЗ И АНТИМИКРОБНА АКТИВНОСТ НА ПИРАЗОЛОВИ ПРОИЗВОДНИ ПОЛУЧЕНИ ЧРЕЗ 1,3-ДИПОЛЯРНО ЦИКЛОПРИСЪЕДИНЯВАНЕ НА НИТРИЛИМИНИ И ЕТИЛАЦЕТОАЦЕТАТ

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(Резюме)

Етилацетоацетат реагира с нитрилимини получени in situ чрез каталитична дехидрогениране на дифенилхидразон в присъствие на хлорамин-Т (САТ) до получаване на съответни региселективни циклоадукти с добив 80%. Структурата на тези съединения е охарактеризирана с ИЧС, 1Н ЯМР, 13С ЯМР, массспектрометрия и елементен анализ. Всички пиразолови производни са изпитани за техните антибактериална и антитубична активности.