Med Chem Res (2012) 21:2702–2708
DOI 10.1007/s00044-011-9796-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization and antimicrobial activity of novel ethyl 1-(N-substituted)-5-phenyl-1*H*-pyrazole-4-carboxylate derivatives

B. Chandrakantha · Arun M. Isloor · Prakash Shetty · Shrikrishna Isloor · Shridhar Malladi · Hoong Kun Fun

Received: 30 January 2011 / Accepted: 10 September 2011 / Published online: 22 September 2011

Abstract In the present study, a novel series of Pyrazole derivatives **(3a–m)** were synthesized by condensing ethyl-3-(dimethylamino)-2-(phenylcarbonyl)prop-2-enoate with different aromatic and aliphatic hydrazines. These newly synthesized compounds were characterized by NMR, mass spectral, IR spectral studies as well as by C, H, and N analyses. All the newly synthesized compounds were screened for their antibacterial properties against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa*. Among the screened samples, **3c**, **3f**, **3k,** and **3l** have showed excellent antibacterial activity against all the tested bacterial strains as compared to the standard drug Ceftriaxone. Few of the compounds were found to be biologically potent. Molecular structure of compound **3i** was confirmed by single crystal X-ray analysis.

B. Chandrakantha
Department of Chemistry, Manipal Institute of Technology, Manipal University, Manipal, India

A. M. Isloor (✉) · S. Malladi
Department of Chemistry, Organic Chemistry Division, National Institute of Technology, Surathkal, Karnataka 575 025, India
e-mail: isloor@yahoo.com

P. Shetty
Department of Printing, Manipal Institute of Technology, Manipal University, Manipal, India

S. Isloor
Department of Microbiology, Veterinary College, Veterinary University, Hebbal, Bangalore, India

H. K. Fun
X-ray Crystallography Unit, School of Physics, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia

Keywords Pyrazole · Antibacterial study · MIC

Introduction

Pyrazoles and their derivatives play an important role in medicinal chemistry research. Several derivatives of pyrazole are of pharmaceutical interest due to their analgesic power (Hall *et al.*, 2008; Isloor *et al.*, 2000). Among them, derivatives of 5-isopyrazolone and pyrazolidine-3,5-dione (Deng *et al.*, 2008) are worth noting due to their clinical interest. Several, substituted pyrazolines are reported to possess moderate antibacterial and antifungal activities (Soliman *et al.*, 2001; Isloor *et al.*, 2009) Besides, a number of nitrofurylpyrazoline derivatives were found to possess antibacterial activity (Ragavan *et al.*, 2010; Premsai Rai *et al.*, 2009; Holla *et al.*, 2000). Pyrazole molecules also exhibits anticancer, anti-inflammatory (Sunil *et al.*, 2009), antidepressant, anticonvulsant (Isloor *et al.*, 2000), and anti HIV properties (Kelekci *et al.*, 2007). The incorporation of aryl system into the pyrazole ring enhances the biological activities to a great extent. The presence of different substituents, both on the pyrazole ring and on the phenyl ring, can severely modify the biological properties of such molecules (El-Sabbagh *et al.*, 2009; Bernard *et al.*, 1985). During the past few years, considerable evidence has been accumulated to demonstrate the efficacy of pyrazole derivatives. Prompted by these observations and in continuation of our search on biologically active heterocycles (Kalluraya *et al.*, 2004; Chandrakantha *et al.*, 2010; Isloor *et al.*, 2010), we describe in this article the synthesis of novel pyrazole derivatives carrying aryl ring system, by condensing ethyl-3-(dimethylamino)-2-(phenylcarbonyl)prop-2-enoate with different aromatic/aliphatic hydrazines.

Results and discussion

Synthesis and characterization

The reaction between ethylbenzoylacetate (**1**) and *N*,*N* dimethylformamide dimethyl acetal for 18 h under reflux condition and further on distilling excess of acetal under reduced pressure, yielded yellow colored ethyl-3-(dimethylamino)-2-(phenylcarbonyl)prop-2-enoate **(2)**. Further reaction between ethyl-3-(dimethylamino)-2-(phenylcarbonyl) prop-2-enoate **(2)** with different series of aromatic/aliphatic hydrazines under reflux, yielded whitish-yellowish crystalline solid **(3)** in reasonable yield. Synthetic route has been presented in Scheme 1.

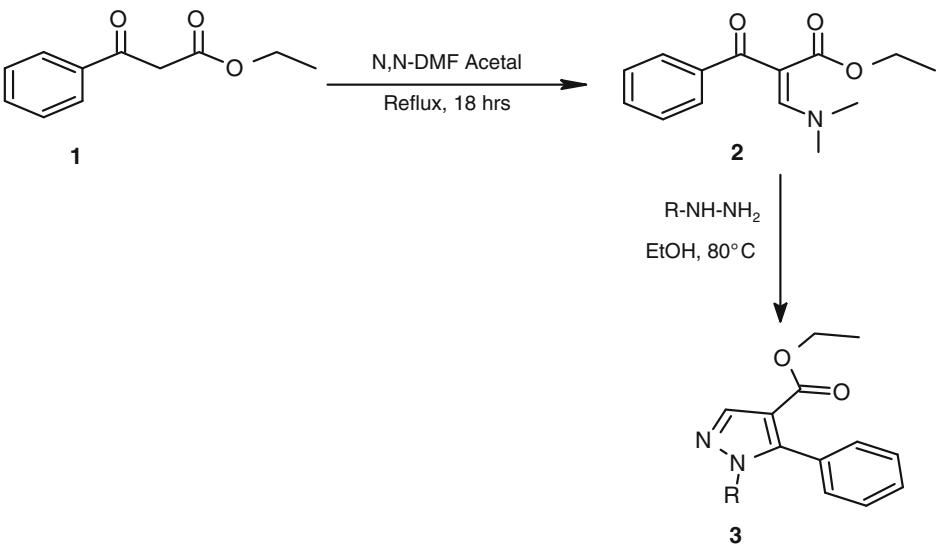
Formation of the ethyl-1-(N-substituted)-5-phenyl-1*H*-pyrazole-4-carboxylate derivatives was confirmed by recording their IR, ^{1}H-NMR, ^{13}C-NMR, mass spectra, and by single crystal X-ray analysis. IR spectrum of compound **3a** showed absorption at 3500 and 2990 cm^{-1}, which is due to the aromatic stretching. Absorption band at 1600 cm^{-1} is due to C=N of pyrazole ring, a band at 1509 cm^{-1} is due to C=C, absorptions at 1230 cm^{-1} and at 1700 cm^{-1} are due to the C–O, C=O stretch of ester, respectively. The ^{1}H-NMR of spectrum of **3a** showed a broad singlet near δ 13.3 for carboxylic group in the phenyl ring and a singlet at δ 8.23, which is due to the pyrazole –CH; a doublet observed in the region of δ 7.88–7.86 is due to the two aromatic protons. Similarly, another multiplet observed in the range of δ 7.43–7.28 is due to the seven protons of the aromatic ring, while a multiplet and triplet at the regions δ 4.13–4.08 and δ 1.12–1.089 correspond to $–CH_2$ and $–CH_3$ of ethyl ester functional group, respectively. The mass spectrum of compound **3a** showed the molecular ion peak at *m/z* 337, which is in agreement with the molecular formula $C_{19}H_{16}N_2O_4$. Similarly, the spectral values for all the compounds and C, H, and N analyses are given in the experimental part. Also, the single crystal X-ray analysis of **3j** further confirmed the structure of the synthesized compounds. Figure 1 shows crystal structure of compound **3i**. The detailed spectral study has been given under the experimental and characterization part.

All the compounds were screened for their possible antibacterial activity against *S. aureus, B. subtilis, E. coli,* and *P. aeruginosa*. Compounds **3c**, **3f**, **3k**, and **3l** have shown excellent antibacterial activity against all the tested bacterial strains as compared with the standard drug Ceftriaxone, which was active at 3.125, 1.6125, 1.6125, and 1.6125 μg/ml against *S. aureus, B. subtilis, E. coli*, and *P. aeruginosa* strains, respectively. Interestingly all these active compounds are halogen-substituted derivatives, which is responsible for the enhanced activity of the compounds.

Experimental

Chemistry

All the chemicals were procured from Aldrich Co, India and were used without any further purification. Reactions were monitored, and purity of the products was checked by TLC which was performed on MERCK 60F-254 silica gel plates. Melting points were determined using BUCHI Melting point B-545 instrument. The IR spectra (in KBr pellets) were recorded on NICOLET 6700FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded on BRUKER (400 MHz) spectrometer in DMSO-d_6 solvent. Mass spectra were recorded on LC–MS-Agilent 1100 series with MSD (Ion trap) using 0.1% aqueous TFA in acetonitrile system on C18-BDS column for 10 min duration. The



Scheme 1 Synthetic route for the pyrazole derivatives. R = Phenyl, 4-carboxyphenyl, 2-carboxyphenyl, 4-CF_3-phenyl, 4-tert-butylphenyl, 4-fluorophenyl, 2-bromophenyl, Quinolinyl, 4-tolyl, tertbutyl, Methyl, 2,4-dicholorophenyl, Piperidyl, and Cyclohexyl

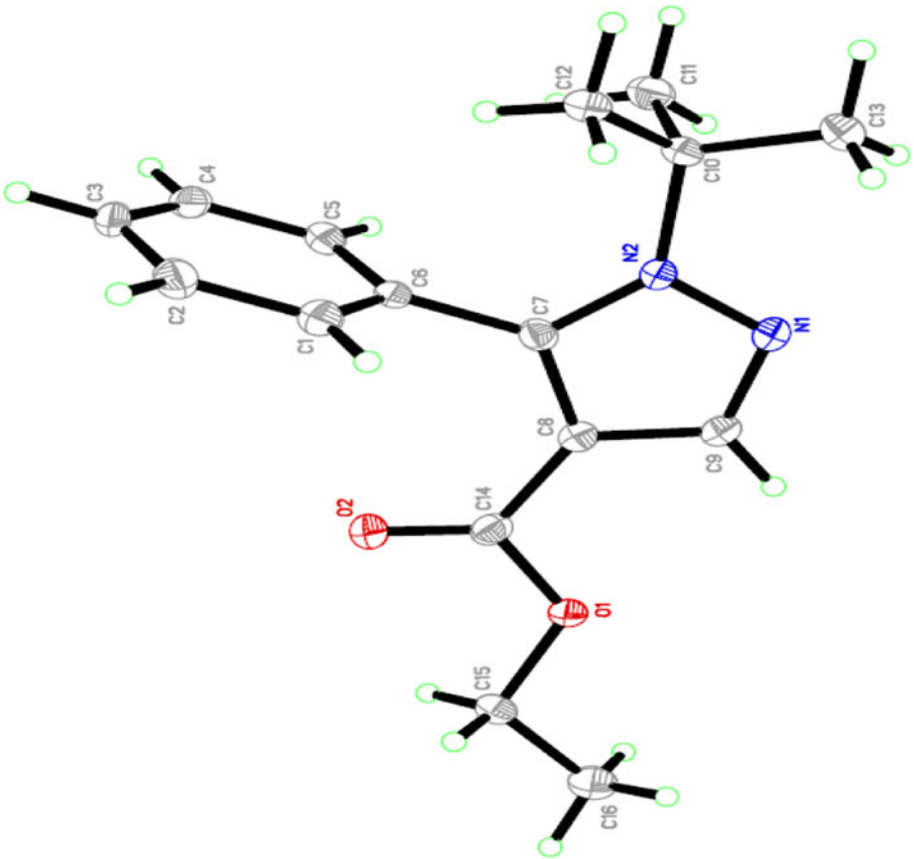


Fig. 1 X-ray crystal structure of compound **(3i)**

elemental analysis was performed using THERMO Finningan FLASH EA 1112 CHN analyzer. Single crystal X-ray analyses were performed using Bruker APEX2 instrument. Column chromatography was performed on silica gel (60–120 mesh) supplied by Acme Chemical Co.(India) for compound purification.

*General procedure for the preparation of ethyl-3-(dimethylamino)-2-(phenylcarbonyl)prop-2-enoate **(2)***

A mixture of ethylbenzoylacetate (**1**) (10 g, 0.0520 mol) and *N*,*N* dimethyl formamide dimethyl acetal (30.9 g, 0.26 mol) was heated to reflux for 18 h. The excess of acetal was distilled off under reduced pressure, and the residue was purified by column chromatography using 60–120 silica gel mesh size using chloroform and methanol as an eluent to give yellow solid.(**2**) (11 g, 85%) with melting point 65–70°C.

*General procedure for preparation of different substituted pyrazole derivatives **(3a–n)***

To a solution of ethyl-3-(dimethylamino)-2-(phenylcarbonyl) prop-2-enoate **(2)** (1.0 eq) in different series of aromatic/aliphatic hydrazines (1.1 eq) were refluxed with absolute ethanol (10 vol) for 2 h, and the excess of solvent was evaporated under reduced pressure. The residue was washed with 1.5 N HCl, and the solid separated was filtered and dried under vacuum. The solid obtained was purified by column chromatography using silica gel 60–120 mesh size and petroleum ether:ethyl acetate as eluent to afford different N-substituted-5-phenyl-1*H*-pyrazole-4-ethyl carboxylate as white and pale yellow crystalline solid. (**3a**–**3m**) in 65–85% yield.

*4-[4-(Ethoxycarbonyl)-5-phenyl-1H-pyrazol-1-yl] benzoic acid **(3a)***

(TLC, Pet-ether/EtOAc, 1:1, $Rf = 0.2$), Yield 75%; white solid.; m.p. 105–110°C; ^{1}H-NMR (DMSO-d_6, 24°C): δ 13.13 (bs, 1H, –COOH group), 8.23 (s, 1H, Pyrazole –CH), 7.88–7.86 (d, 2H, $J = 8.56$ Hz, Ar–H), 7.43–7.28 (m, 7H, Ar–H), 4.13–4.08 (q, 2H), 1.12–1.08 (t, 3H, $J = 7.12$ Hz). ^{13}C-NMR (DMSO-d_6) 165.15, 162.95, 145.33, 142.07, 139.00, 131.67, 130.44, 129.49, 128.78, 128.34, 127.98, 126.01, 113.62., 59.78, 13.83. MS: $m/z = 337.3$ (M^+); Method: A-0.1% HCOOH, B-MEOH, Coloumn: Atlantis dC18 (50 × 4.6 mm) 5 mm. Flow rate 1.0 ml/min. IR (KBr) cm^{-1}: 3500, 2990 (Ar–H), C=N (1600-stretch of Pyrazole ring), C=C (1509), C–O (1230), C=O

(1700-stretch of ester); Anal. Calcd. (Found) for $C_{19}H_{16}N_2O_4$: C, 67.85 (67.66); H, 4.79 (4.88); N, 8.33 (8.56).

4-[3-(Ethoxycarbonyl)-5-phenyl-1H-pyrazol-1-yl]benzoic acid ***(3b)***

(TLC, Pet-ether/EtOAc, 1:1, $Rf = 0.40$), Yield 82%; white solid.; m.p. 90–100°C; ^{1}H-NMR (DMSO-d_6): δ 13.17 (bs, 1H, –COOH), 8.21 (s, 1H, pyrazole –CH), 7.88–7.86 (d, 1H, $J = 7.44$ Hz, Ar–H), 7.78 (s, 1H, Ar–H), 7.47–7.29 (m, 7H, Ar–H), 4.13–4.07 (q, 2H), 1.15–1.08 (t, 3H, $J = 7.04$ Hz).^{13}C-NMR (DMSO-d_6) 166.15, 161.95, 145.33, 142.07, 139.00, 131.67, 130.44, 129.49, 129.32, 129.20, 128.78, 128.34, 127.98, 126.01, 113.62., 59.68, 13.93. MS: $m/z = 337.3$ (M^+); Method: A-0.1% HCOOH, B-MEOH, Column: Atlantis dC18 (50 × 4.6 mm) 5 mm. Flow rate 1.0 ml/min. IR (KBr) cm^{-1}: 3453, 2996 (Ar–H), C=N (1634-stretch of Pyrazole ring), C=C (1560), C–O (1300), C=O (1642-stretch of ester); MS: $m/z = 337.3$ (M^+); Anal. Calcd.(Found) for $C_{19}H_{16}N_2O_4$: C, 67.85 (67.80); H, 4.79 (4.82); N, 8.33 (8.42).

Ethyl 5-phenyl-1-[4-(trifluoromethyl)phenyl]-1H-pyrazole-4-carboxylate ***(3c)***

(TLC, Pet-ether/EtOAc, 8:2, $Rf = 0.5$), Yield 78%; white solid; m.p. 98–110°C; ^{1}H-NMR (DMSO-d_6): δ 8.25 (s, 1H, Pyrazole -CH), 7.75–7.73 (d, 2H, $J = 8.48$ Hz, Ar–H), 7.44–7.39 (m, 5H, Ar–H), 7.36-7.31 (t, 2H, $J = 11.6$ Hz, Ar–H), 4.13–4.08 (q, 2H), 1.12–1.08 (t, 3H, $J = 7.08$ Hz) MS: $m/z = 361.3$ (M^+) Method: A- 0.1%TFA, B-MEOH, Column: XBridge C18 (50 × 4.6 mm) 3.5 mm. Flow rate 2.0 ml/min. IR (KBr) cm^{-1}: 3553, 2896 (Ar–H), C=N (1658-strecth of Pyrazole ring), C=C (1567), C–O (1412), C=O (1650-stretch of ester);; Anal. Calcd. (Found) for $C_{19}H_{15}F_3N_2O_2$: C, 63.33 (63.33); H, 4.20 (4.24); N, 7.77 (7.72).

Ethyl 1-(4-tert-butylphenyl)-5-phenyl-1H-pyrazole-4-carboxylate ***(3d)***

(TLC, Pet-ether/EtOAc, 8:2, $Rf = 0.5$) Yield 85%; white solid; m.p. 125–130°C; ^{1}H NMR (DMSO-d_6): δ 8.15 (s, 1H, Pyrazole –CH), 7.38–7.32 (m, 5H, Ar–H), 7.29–7.28 (d, 2H, $J = 7.64$ Hz, Ar–H), 7.14–7.12 (d, 2H, $J = 8.52$ Hz, Ar–H), 4.11–4.06 (q, 2H), 1.22(s, 9H, tert-buty) 1.10–1.07 (t, 3H, $J = 7.08$ Hz) MS: $m/z = 349.4$ (M^+) Method: A- 0.1%TFA, B-MEOH, Column: XBridge C18 (50 × 4.6 mm) 3.5 mm. Flow rate 2.0 ml/min. IR (KBr) cm^{-1}: 3560, 2885 (Ar–H), C=N (1670-stretch of Pyrazole ring), C=C (1585), C–O (1415), C=O (1660-stretch of ester); Anal. Calcd.(Found) for $C_{22}H_{24}N_2O_2$: C, 75.83 (75.63); H, 6.94 (7.00); N, 8.04 (7.95).

Ethyl 1-(4-fluorophenyl)-5-phenyl-1H-pyrazole-4-carboxylate ***(3e)***

(TLC, Pet-ether/EtOAc, 9:1, $Rf = 0.3$) Yield 76%; white solid; m.p. 140–150°C; ^{1}H-NMR (DMSO-d_6): δ 8.17 (s, 1H, pyrazole –CH), 7.38–7.32 (m, 3H, Ar–H), 7.29–7.27 (m, 4H, Ar–H), 7.27–7.18 (m, 2H, Ar–H), 4.12–4.07 (q, 2H), 1.11–1.08 (t, 3H, $J = 7.08$ Hz). MS: $m/z = 311.3$ (M^+) Method: A- 0.1%TFA, B-MEOH, Column: XBridge C18 (50 × 4.6 mm) 3.5 mm. Flow rate 2.0 ml/min IR (KBr) cm $^{-1}$: 3570, 2865 (Ar–H), C=N (1690-stretch of Pyrazole ring), C=C (1575), C–O (1445), C=O (1670-stretch of ester). Anal. Calcd. (Found) for $C_{18}H_{15}FN_2O_2$: C, 69.67 (69.71); H, 4.87 (4.87); N, 9.03 (9.03).

Ethyl 1-(2-bromophenyl)-5-phenyl-1H-pyrazole-4-carboxylate ***(3f)***

(TLC, Pet-ether/EtOAc, 9:1, $Rf = 0.3$) Yield 80%; white solid.; m.p. 80–90°C; ^{1}H-NMR (DMSO-d_6): δ 8.2(s, 1H, Pyrazole –CH), 7.68–7.66 (d, 1H, $J = 7.9$ Hz, Ar–H), 7.60–7.58 (d, 1H, $J = 7.76$ Hz, Ar–H), 7.45–7.41 (m, 1H, Ar–H), 7.38–7.28 (m, 6H, Ar–H), 4.13–4.08(q, 2H), 1.12–1.09 (t, 3H, $J = 7.08$ Hz). ^{13}C-NMR (DMSO-d_6) 162.04, 146.74, 141.84, 137.94, 133.01, 131.59, 130.77, 130.13, 129.13, 128.39, 127.82, 127.61, 121.59, 112.55, 59.67, 13.95. MS: $m/z = 371.3$ (M^+) Method: A- 0.1%TFA, B-MEOH, Column: XBridge C18 (50 × 4.6 mm) 3.5 mm. Flow rate 2.0 ml/min; IR (KBr) cm^{-1}: 3550, 2855(Ar–H), C=N (1680-stretch of Pyrazole ring), C=C (1585), C–O (1485), C=O (1680-stretch of ester); Anal. Calcd. (Found) for $C_{18}H_{15}BrN_2O_2$: C, 58.24 (58.55); H, 4.07 (4.15); N, 7.55 (7.62).

Ethyl 5-phenyl-1-quinolin-2-yl-1H-pyrazole-4-carboxylate ***(3g)***

(TLC, Pet-ether/EtOAc, 7:3, $Rf = 0.4$) Yield 82%; pale yellow solid.; m.p. 150–160°C; ^{1}H-NMR (DMSO-d_6): δ 8.52–8.50 (d, 1H, $J = 8.76$ Hz, quinoline H), 8.2 (s, 1H, Pyrazole –CH), 8.01–7.99 (d, 1H, $J = 8.08$ Hz, Ar–H), 7.78–7.73 (d, 1H, $J = 8.7$ Hz, Ar–H), 7.70–7.68 (t, 1H, $J = 7.08$ Hz, Ar–H), 7.62–7.58 (t, 1H, $J = 7.96$ Hz, Ar–H), 7.47–7.45 (d, 1H, $J = 8.36$ Hz), 7.33–7.30 (m, 5H, Ar–H) 4.15–4.09 (q, 2H), 1.14–1.05 (t, 3H, $J = 7.12$ Hz). ^{13}C-NMR (DMSO-d_6) 161.91, 150.06, 145.98, 145.25, 142.36, 139.31, 130.63, 130.20, 129.45, 128.60, 128.15, 127.88, 127.40, 127.28, 126.87, 117.12, 114.33, 59.77, 13.90. MS: $m/z = 344.3$ (M^+) Method: A- 0.1%TFA, B-MEOH, Column: XBridge C18 (50 × 4.6 mm) 3.5 mm. Flow rate 2.0 ml/min. IR (KBr) cm^{-1}: 3530, 2835 (Ar–H), C=N (1630-stretch of Pyrazole ring), C=C (1535), C–O (1455), C=O (1680-stretch of ester); Anal. Calcd. (Found)

for $C_{21}H_{17}N_3O_2$: C 73.45 (73.45), H 4.99 (4.97), N 12.24(12.20).

Ethyl 1-(4-methylphenyl)-5-phenyl-1H-pyrazole-4-carboxylate ***(3h)***

(TLC, Pet-ether/EtOAc, 8:2, $Rf = 0.3$) Yield 80%; pale yellow solid; m.p. 105–115°C; ^{1}H-NMR (DMSO-d_6): δ 8.15 (s, 1H, pyrazole-CH), 7.37–7.31 (m, 3H, Ar–H), 7.27–7.25 (t, 2H, $J = 7.68$ Hz, Ar–H), 7.14–7.12 (d, 1H, $J = 8.321$ Hz, Ar–H), 7.09–7.07 (d, 2H, $J = 8.40$ Hz, Ar–H), 4.11–4.06 (q, 2H), 2.26 (s, 1H, –CH3 1.12–1.09 (t, 3H, $J = 7.08$ Hz). ^{13}C-NMR (DMSO-d_6)162.04, 145.12, 141.55, 137.78, 136.45, 130.40, 129.33, 128.96, 128.65, 127.84, 125.41, 113.17, 59.56, 20.51, 13.94. MS: *m/z* = 307.4 (M^+) Method: A- 0.1% HCOOH, B-MEOH, Column: Atlantis dC18 (50 × 4.6 mm) 5 mm. Flow rate 1.0 ml/min IR (KBr) cm $^{-1}$: 3670, 2855 (Ar–H), C=N (1690-stretch of Pyrazole ring), C=C (1545), C–O (1475), C=O (1640-stretch of ester); Anal. Calcd. (Found) for $C_{19}H_{18}N_2O_2$: C, 74.49 (74.60); H, 5.92 (6.00); N, 9.14 (9.23).

Ethyl 1-tert-butyl-5-phenyl-1H-pyrazole-4-carboxylate ***(3i)***

(TLC, Pet-ether/EtOAc, 8:2, $Rf = 0.2$) Yield 70%; pale yellow solid; m.p. 69–75°C; Molecular structure of the compound has been presented in Fig. 1. ^{1}H-NMR (DMSO-d_6): δ 7.88 (s, 1H, Ar–H), 7.46–7.40 (m, 3H, Ar–H), 7.34–7.31 (m, 2H, Ar–H), 3.93 (q, 2H), 1.35 (s, 9H, tert butyl), 0.94–0.90 (t, $J = 7.12$ Hz, 3H). MS: *m/z* = 273.3 (M^+) Method: A- 0.1% HCOOH, B-MEOH, Column: Atlantis dC18 (50 × 4.6 mm) 5 mm. Flow rate 1.0 ml/min IR (KBr) cm $^{-1}$: 3630, 2835 (Ar–H), C=N (1630-stretch of Pyrazole ring), C=C (1535), C–O (1435), C=O (1630-stretch of ester). Anal. Calcd.(Found) for $C_{16}H_{20}N_2O_2$: C, 70.56 (70.65); H, 7.40 (7.35); N, 10.29 (10.33).

Ethyl 1-methyl-5-phenyl-1H-pyrazole-4-carboxylate ***(3j)***

(TLC, Pet-ether/EtOAc, 9:1, $Rf = 0.23$) Yield 78%; pale yellow solid. m.p. 70–75°C. ^{1}H-NMR (DMSO-d_6): δ 8.33(s, 1H, Pyrazole H), 7.47–7.33 (m, 5H, Ar–H), 4.12–4.06 (q, 2H), 2.16 (s, 3H,-CH3), 1.10–1.06 (t, $J = 7.08$ Hz, 3H). MS: *m/z* = 230.3 (M^+) Method: A- 0.1% HCOOH, B-MEOH, Column: Atlantis dC18 (50 × 4.6 mm) 5 mm. Flow rate 1.0 ml/min IR (KBr) cm^{-1}: 3640, 2825 (Ar–H), C=N (1690-stretch of Pyrazole ring), C=C (1565), C–O (1435), C=O (1700-stretch of ester); Anal. Calcd.(Found) for $C_{13}H_{14}N_2O_2$: C, 67.81 (67.81); H, 6.13 (6.11); N, 12.17 (12.12).

Ethyl 1-(2,4-dichlorophenyl)-5-phenyl-1H-pyrazole-4-carboxylate ***(3k)***

(TLC, Pet-ether/EtOAc, 7:3, $Rf = 0.5$) Yield 84%; pale yellow solid.; m.p. 125–135°C; ^{1}H-NMR (DMSO-d_6): δ 8.22 (s, 1H, pyrazole -H), 8.21–7.4 (m, 1H, Ar–H), 7.4–7.17 (m, 7H, Ar–H), 4.14 (q, 2H), 1.13–1.09 (t, $J = 7.08$ Hz, 3H). ^{13}C-NMR (DMSO-d_6) 161.04, 144.74, 142.84, 136.94, 134.01, 132.59, 131.77, 131.13, 128.13, 129.39, 128.82, 126.61, 123.59, 114.55, 59.77, 13.92. MS: *m/z* = 362.2 (M^+) Method: A- 0.1% HCOOH, B-MEOH, Column: Atlantis dC18 (50 × 4.6 mm) 5 mm. Flow rate 1.0 ml/min; IR (KBr) cm^{-1}: 3620, 2825 (Ar–H), C=N (1620-stretch of Pyrazole ring), C=C (1525), C–O (1425), C=O (1620-stretch of ester); Anal. Calcd. (Found) for $C_{18}H_{14}C_{l2}N_2O_2$: C, 59.85 (59.80); H, 3.91(3.97); N, 7.76 (7.72).

Ethyl 5-phenyl-1-piperidin-4-yl-1H-pyrazole-4-carboxylate ***(3l)***

(TLC, chloroform/methanol, 8:2, $Rf = 0.35$) Yield 81%; pale yellow solid; m.p. 60–70°C; ^{1}H-NMR (DMSO-d6): δ 8.01 (s, 1H, pyrazole H), 7.52–7.49 (m, 3H, Ar–H), 7.41–7.37 (m, 2H, Ar–H), 4.13–4.06 (q, 2H), 3.92 (m, 1H), 3.1–2.9 (m, 3H), 2.8–2.49 (m, 1H), 2.01–1.96 (m, 2H), 1.67–1.64 (m, 1H), 1.33–1.30 (m, 1H), 1.10–1.06 (t, $J = 7.08$ Hz, 3H). ^{13}C-NMR (DMSO-d_6) 162.05, 145.11, 140.43, 129.89, 129.23, 128.62, 127.64, 111.86, 59.24, 54.82, 50.10, 24.44, 13.88. MS: *m/z* = 300.4 (M^+) Method: A- 0.1% HCOOH, B-MEOH, Column: Atlantis dC18 (50 × 4.6 mm) 5 mm. Flow rate 1.0 ml/min; IR (KBr) cm^{-1}: 3620 (Ar–H), C=N (1650-stretch of Pyrazole ring), C=C (1555), C–O (1455), C=O (1650-stretch of ester), Anal. Calcd. (Found) for $C_{17}H_{21}N_3O_2$: C, 68.20 (68.20); H, 7.07 (7.01); N, 14.04 (14.01).

Ethyl 1-cyclohexyl-5-phenyl-1H-pyrazole-4-carboxylate ***(3m)***

(TLC, Pet-ether/EtOAc, 7:3, $Rf = 0.35$) Yield 78%; pale yellow solid; m.p. 85–90°C; ^{1}H-NMR (DMSO-d_6): δ 8.12 (s, 1H, pyrazole H), 7.65–7.25 (m, 5H, Ar–H), 4.13–4.06 (q, 2H), 3.3–3.2 (m, 1H), 3.1–2.5 (m, 2H), 2.45–2.20 (m, 4H), 2.2–1.99 (m, 4H), 1.12–1.07 (t, $J = 7.08$ Hz, 3H). ^{13}C-NMR (DMSO-d_6) 161.05, 144.11, 142.43, 128.89, 128.23, 128.62, 127.84, 11.96, 59.24, 54.82, 54.60, 50.10, 24.44, 13.88. MS: *m/z* = 299.4 (M^+) Method: A- 0.1%TFA, B-MEOH, Column: XBridge C18 (50 × 4.6 mm) 3.5 mm. Flow rate 2.0 ml/min IR (KBr) cm^{-1}: 3630 (Ar–H), C=N (1630-stretch of Pyrazole ring), C=C (1535), C–O (1435), C=O (1630-stretch of ester), Anal. Calcd. (Found) for $C_{18}H_{22}N_2O_2$: C, 72.46 (72.50); H, 7.43 (7.39); N. 9.39 (9.39).

Antimicrobial studies

All the newly synthesized compounds were screened for their antibacterial activity. For this, *S. aureus, B. subtilis, E. coli*, and *P. aeruginosa* microorganisms were employed. Antimicrobial study was assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method (Mackie and McCartney 1989) Several colonies of *S. aureus, B. subtilis, E. coli*, and *P. aeruginosa* were picked off a fresh isolation plate and inoculated in corresponding tubes containing 5 ml of trypticase soya broth. The broth was incubated for 6 h at 37°C until there was visible growth. Mc Farland No.5 standard was prepared by adding 0.05 ml of 1% w/v $BaCl_2.2H_2O$ in Phosphate Buffered saline (PBS) to 9.95 ml of 1% v/v H_2SO_4 in PBS. The growths of all the four cultures were adjusted to Mc Farland No.5 turbidity standard using sterile PBS. This gives a 10^8 cfu/ml suspension. The working inoculums of aforementioned four different microorganisms containing 10^5 cfu/ml suspension was prepared by diluting the 10^8 cfu/ml suspension, 10^3 times in trypticase soya broth.

Preparation of anti-microbial suspension (50 μg/ml)

Dissolved 0.5 mg of each compound in 10 ml of trypticase soya broth to get 50 μg/ml. This suspension was filter sterilized in syringe filters.

Preparation of dilutions

In all, for each of the 14 anti-microbial compounds and standard antimicrobial, i.e., Ceftriaxone, 24 tubes of 5-ml capacity were arranged in four rows with each row containing six tubes. Then, 1.9 ml of trypticase soya broth was added in the first tube in each row and 1 ml in the remaining tubes. Now, 100 μl of filtered anti microbial suspension was added to the first tube in each row and after mixing the content, 1 ml was serially transferred from these tubes to the second tube in each of the rows. The contents in the second tube of each of the rows were mixed and transferred to the third tube in each of the rows. This serial dilution was repeated till the sixth tube in each of the rows. This provided anti-microbial concentrations of 50, 25, 12.5, 6.25, 3.125, and 1.6125 μg/ml in the first to sixth tube, respectively, in each row. Finally, 1 ml of 10^5 cfu/ml of *S. aureus, B. subtilis, E. coli*, and *P. aerogenosa* suspension were added to the first, second, third, and fourth rows of tubes, respectively. Along with the test samples and Ceftriaxone (standard), the inoculums control (without antimicrobial compound) and broth control (without antimicrobial compound and inoculum) were maintained. All the test samples and control tubes were then incubated for 16 h at 37°C.

Interpretation

After incubation, the tubes showing no visible growth were considered to be representing the MIC. The details of results are furnished in Table 1. Inoculums control showed visible growth, whereas the broth control showed no growth.

Conclusions

A novel series of ethyl-1-(N-substituted) 5-phenyl-1*H*-pyrazole-4 carboxylate derivative were synthesized and

Table 1 Antibacterial data for the newly synthesized pyrazole derivatives in MIC (μg/ml)

Comp.	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
3a	50.00	Growth in all concentrations	Growth in all concentrations	25.00
3b	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations
3c	3.125	1.6125	1.6125	1.6125
3d	Growth in all concentrations	12.50	Growth in all concentrations	12.50
3e	6.250	3.125	1.6125	1.6125
3f	3.125	1.6125	1.6125	1.6125
3g	3.125	3.125	6.250	3.125
3h	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations
3i	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations
3j	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations
3k	1.6125	1.6125	1.6125	3.250
3l	3.125	3.125	1.6125	1.6125
3m	6.250	6.250	12.50	6.250
Ceftriaxone (Standard)	3.125	1.6125	1.6125	1.6125
Inoculum	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations	Growth in all concentrations
Broth control	No growth	No growth	No growth	No growth

characterized by IR, ^{1}H-NMR and mass spectrometry studies. Molecular structure **3i** was also confirmed by single crystal X-ray analysis. All the synthesized compounds were screened for their antibacterial activity by serial dilution (MIC) method. As regards the relationships between the structure of the heterocyclic scaffold and the detected antibacterial properties, it showed varied biological activity. Among the screened samples, **3c**, **3f**, **3k**, and **3l** have shown excellent antibacterial activity against all the tested bacterial strains as compared with the standard drug Ceftriaxone, which was active at 3.125, 1.6125, 1.6125, and 1.6125 μg/ml against S. aureus, B.subtilis, E.coli, and *P.aeruginosa* strains, respectively. Interestingly all these active compounds are halogen-substituted derivatives, which is responsible for the enhanced activity. Compound **3e**, which is fluorine substituted compound, has shown significant activity. Compounds **3g** and **3l**, which have quinolin and piperidin substituents, respectively, also have shown significant antibacterial activity against all bacterial strains. All the remaining compounds have exhibited poor antibacterial activity. In our recently published study (Vijesh *et al.*, 2011), pyrazole incorporated imidazoles were shown to have significant antibacterial activity. In conclusion, pyrazole moiety is one of the active components present in all the synthesized molecules, which has shown significant activity in the presence of halogen substituent.

Acknowledgment AMI is thankful to Board of Research in Nuclear Sciences, Department of Atomic Energy, Government of India for the "Yong Scientist" award.

References

Bernard AM, Cocco MT, Maccioni A, Plumitallo A (1985) Phytotoxic activity in pyrazole derivatives 1. Il Farm Ed Sci 40:259–271

Chandrakantha B, Shetty P, Nambiyar V, Isloor N, Isloor AM (2010) Synthesis, characterization and biological activity of some new 1,3,4-oxadiazole bearing 2-flouro-4-methoxy phenyl moiety. Eur J Med Chem 4:1206–1210

Deng G, Weihua Li, Shen J, Jiang H, Chen K, Liu H (2008) Pyrazolidine-3,5-dione derivatives as potent non-steroidal agonists of farnesoid X receptor: virtual screening, synthesis, and biological evaluation. Bioorg Med Chem Lett 18:5497–5502

El-Sabbagh OI, Baraka MM, Ibrahim SM, Pannecouque C, Andrei G, Snoeck R, Balzarin J, Rashad AA (2009) Synthesis and antiviral activity of new pyrazole and thiazole derivatives. Eur J Med Chem 44:3746–3753

Hall A, Billinton A, Brown SH, Clayton NM, Chowdhury A, Gerald MP, Goldsmith GP, Hayhow TG, Hurst DN, Kilford IR, Naylor A, Passingham B (2008) Non-acidic pyrazole EP_1 receptor antagonists with in vivo analgesic efficacy. Bioorg Med Chem Lett 18:3392–3399

Holla BS, Akberali PM, Shivananda MK (2000) Synthesis and antibacterial properties of arylfuryl-Δ^2-pyrazolines. Il Farmaco 55:256–263

Isloor AM, Kalluraya B, Rao M (2000) Synthesis of 3-aryl-4-(substituted Pyrazolidene hydrazine-4-thiazolyl) sydnones as possible analgesic and anticonvulsant agents. J Saudi Chem Soc 4:265–270

Isloor AM, Kalluraya B, Shetty P (2009) Regioselective reaction: synthesis, characterization and pharmacological studies of some new Mannich bases derived from 1,2,4-triazoles. Eur J Med Chem 44:3784–3787

Isloor AM, Kalluraya B, Pai KS (2010) Synthesis, characterization and biological activities of some new benzo[b]thiophene derivatives. Eur J Med Chem 45:825–830

Kalluraya B, Jagadeesha RL, Isloor AM (2004) Synthesis and pharmacological activity of some 4-(substituted)-2-[4-arylhydrazono-3-methyl-5-oxo-2-pyrazolin-1-yl] thiazoles. Indian J Het Chem 13:245–248

Kelekci NG, Yabanoglu S, Kupeli E, Salgın U, Ozgen O, Ucar G, Yeşilada E, Kendi E, l Yesilada A (2007) A new therapeutic approach in Alzheimer disease: some novel pyrazole derivatives as dual MAO-B inhibitors and antiinflammatory analgesics. Bioorg Med Chem 15:5775–5786

Mackie, McCartney (1989) Practical medical microbiology, vol. 13, Churchill Livingstone, UK

Premsai Rai N, Narayanaswamy VK, Shashikanth S (2009) Synthesis, characterization and antibacterial activity of 2-[1-(5-chloro-2-methoxy-phenyl)-5-methyl-1H-pyrazol-4-yl]-5-(substituted-phenyl)-[1,3,4]oxadaizole. Eur J Med Chem 44:4522–4527

Ragavan V, Vijay Kumar V, Kumari SN (2010) Synthesis and antimicrobial activity of novel 1,5-diaryl pyrazole. Eur J Med Chem 45:1173–1180

Soliman R, Habib NS, Ashour FA, El-Taiebi M (2001) Synthesis and antimicrobial activity of novel pyrazole, pyrazoline, pyrazolinone and pyrazolidinedione derivatives of benzimidazole. Boll Chim Farm 140:140–148

Sunil D, Isloor AM, Shetty P (2009) Synthesis, characterization and anticancer activity of 1,2,4-Triazolo[3,4-b]-1,3,4-thiadiazoles on HepG2 cell lines. Der Pharma Chem 1:19–26

Vijesh AM, Isloor AM, Telkar S, Peethambar SK, Rai S, Isloor N (2011) Synthesis, characterization and antimicrobial studies of some new pyrazole incorporated imidazole derivatives. Eur J Med Chem 46:3531–3536