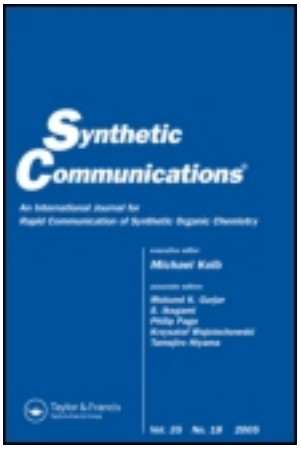

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Synthesis of 3-Substituted Pyrazole Derivatives by Mixed Anhydride Method and Study of Their Antibacterial Activities

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SYNTHESIS OF 3-SUBSTITUTED PYRAZOLE DERIVATIVES BY MIXED ANHYDRIDE METHOD AND STUDY OF THEIR ANTIBACTERIAL ACTIVITIES

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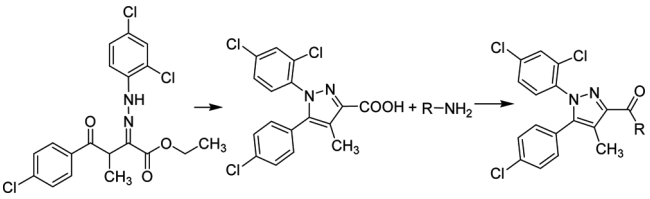
GRAPHICAL ABSTRACT



Abstract *A convenient synthesis of 3-substituted pyrazole derivatives by a mixed anhydride method using* i-*butylchloroformate and* N-*methylmorpholine at −20 °C in tetrahydrofuran and study of in vitro antibacterial activities of the prepared compounds against* Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa*, and* Proteus valguris *by agar-diffusion method were carried out. The results suggested that the products* ***4a***, ***4b***, *and* ***4c*** *exhibited moderate to feeble inhibition against all test bacteria at greater concentration but* ***4d*** *was best against* Staphylococcus epidermidis *(22 mm) and worst against* Pseudomonas aeruginosa *(16 mm) at the greatest concentration (2.5 mg/ml), and the activities decreased with decrease in concentration.*

[Supplementary materials are available for this article. Go to the publisher's online edition of Synthetic Communications® *for the following free supplemental resource(s): Full experimental and spectral details.]*

Keywords Acid anhydride; antibacterial activity; pyrazole

INTRODUCTION

Pyrazole scaffold and its derivatives, a class of well-known nitrogen-containing heterocyclic compounds, play vital roles in biologically active compounds and therefore represent an interesting template for combinatorial as well as medicinal chemistry.[1–5] Pyrazoles are one of the oldest classes of bioactive compounds, which are principally used in medicine and have enormous potential as pharmaceutical

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agents because of their biological activities such as anti-inflammatory,[6–8] antipyretic,[9,10] antiarrhythmic,[11] anticonvulsant,[12,13] antibacterial,[14–17] endocrinological, and antihyperglycaemic[18] activities and are also found to exhibit HIV-1 reverse transcriptase and IL-1 synthesis inhibition.[19] Certain alkyl pyrazoles have shown significant analgesic, antipyretic, bacteriostatic, bactericidal, and fungicidal activities.[20–23] Benzopyrano[4,3]-pyrazoles are found to be potential pharmaceutical agents because of their biological activities such as antimicrobial, anti-inflammatory, and immunomodulator activity.[24–27] Substituted pyrazoles have pronounced sedative action on the central nervous system.[28] The recent success of pyrazole COX-2 inhibitor has further highlighted the importance of these hetrocycles in medicinal chemistry. The synthesis of pyrazole and its analogs has been a subject of consistent interest because of the wide range of applications for such heterocycles in the pharmaceutical and agrochemical industries. Pyrazole-containing compounds have inhibitors and deactivators of liver alcohols dehydrogenase and oxidoreductase. Though many syntheses have been developed for pyrazole derivatives because of their great importance, designing facile synthetic routes remains as an active research area.

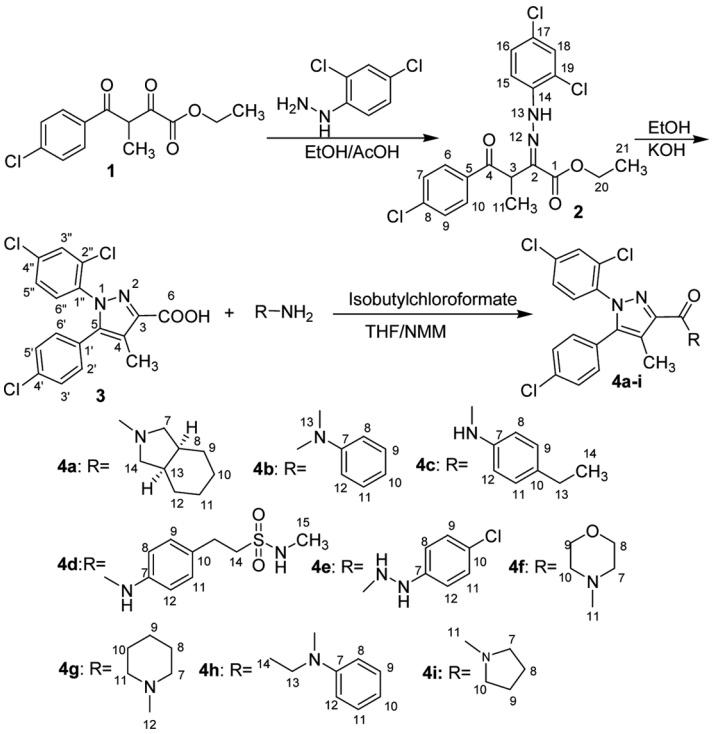
In view of these facts, the aim of the present study was to obtain pyrazole derivatives as antibacterial agents by the mixed anhydride method.

RESULTS AND DISCUSSION

The route used for the synthesis of the pyrazole derivatives **4** assessed in this study is shown in Scheme 1. The reaction of equimolar amount of 4-(4-chlorophenyl)-3-methyl-2,4-dioxo-butyric acid ethyl ester **1** with 2,4-dichlorophenylhydrazine in ethanol (10 times) in the presence of a catalytic amount of acetic acid under reflux condition furnished 4-(4-chloro-phenyl)-2-[(2,4-dichloro-phenyl)-hydrazono]-3-methyl-4-oxo-butyric acid ethyl ester **2** with more than 90% yield. Compound **2** was formed regioselectively becauseof high electron deficiency of carbonyl carbon at C-2 as compared to other two carbonyl carbon at C-1 and C-4. The reactivity at C-1 is decreased by its conjugation with ethoxy group whereas the electropositive character at C-4 relatively lessens because of the +M effect of *p*-chlorophenyl group.

The ester derivative **2** on subsequent cyclization followed by hydrolysis with potassium hydroxidein refluxing ethanol (5 times) gave the precursor 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid **3** in 90% yield (**4g**). The novel pyrazole derivatives **4** were prepared by the reaction of 5-(4-chloro-phenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid **3** with different amines by novel mixed anhydride method using isobutylchloroformate[29–31] in tetrahydrofuran (THF) and in the presence of *N*-methylmorpholine (NMM) at $-20\,^{\circ}C$ with more than 95% yield, which has the advantage of giving better yield than the earlier acid chloride method using thionyl chloride and *N,N*-dimethylformamide in toluene at reflux temperature. To our delight, under these conditions, the reactions proceeded smoothly and a variety of the desired pyrazole products **4a–i** were obtained in good yields (Table 1). The probable mechanism for acid anhydride method is outlined in Scheme 2.

The structures of the synthesized compounds were confirmed by analytical and spectral data (IR, NMR, mass). The IR spectrum of compound **2** illustrates a band at

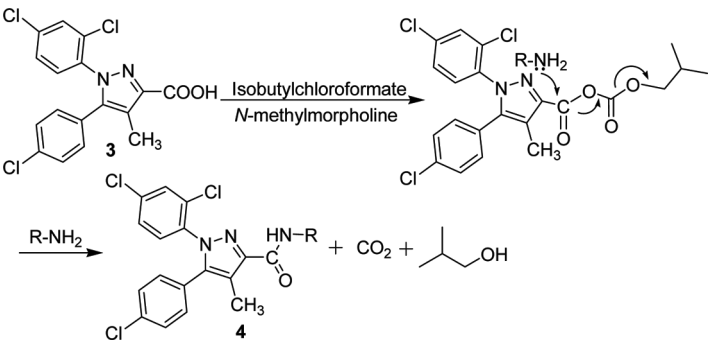


Scheme 1. Synthesis of pyrazole derivatives **4**.

3227 cm^{-1} for NH stretching frequency along with the bands at 1694 and 1681 cm^{-1} assignable to carbonyl groups. ^{1}H NMR of compound **2** shows a sharp singlet at δ 12.41 for NH proton. C=O peaks of **2** in ^{13}C NMR spectrum appears at δ 162.36 (C-1) and 199.17 (C-4). Appearance of a carbonyl peak at δ 1687 cm^{-1} in the IR spectrum of **3** and a broad singlet at δ 8.49 for OH proton along with the disappearance of NH peak in the ^{1}H NMR spectrum indicates the formation of **3**. ^{13}C NMR spectrum of **3** shows a carbonyl peak at δ 164. The IR and NMR spectrum of **4a**, **4b**, and **4f–i** exhibited the disappearance of OH signal. Also, the PMR spectrum of **4a**, **4b**, and **4f–i** showed the appearance of a singlet or quartetfor methyl protons and

Table 1. Synthesis of compound **4** from **3**

Compound	Time (min)	Yield (%)	M (°C)
4a	20	95	185–195
4b	20	96	187–192
4c	20	95	190–195
4d	20	95	188–195
4e	20	96	181–185
4f	20	97	172–177
4g	20	96	174–181
4h	20	96	189–194
4i	20	95	165–170



Scheme 2. Plausible mechanism for the formation of pyrazole derivatives **4**.

multiplet or triplet for methylene protons at δ 3.54–3.95 and 1.25–3.80, respectively. In the IR spectrum of pyrazole derivatives **4c–4e** absorbs strongly at 3221–3385 cm^{-1}, assignable to -NH- group. Moreover, the PMR spectrum of **4c–4e** showed the appearance of broad singletsor quartet at δ 8.55–8.69 or 4.41, respectively. All the detailed spectral data were given in the experimental section.

ANTIMICROBIAL ACTIVITY

All the synthesized compounds were screened for their antibacterial activity in vitro against two Gram-positive bacteria (*Staphylococcus epidermidis and Bacillus subtilis*) and two Gram-negative bacteria (*Pseudomonas aeruginosa* and *Proteus valguris*), which were obtained from the microbial type culture collection and Gene Bank (MTCC). The MTCC is a modern facility housed at the Institute of Microbial Technology (IMTECH), Chandigarh, India, and maintained in usual laboratory conditions.

For preliminary screening the antimicrobial tests were carried out by the agar-diffusion method. The turbidity of bacterial cultures in broth media was adjusted with sterile saline (0.9% w/v) according to 0.5 McFarland turbidity standards for the preparation of the inoculums. Mueller–Hinton agar medium (Hi-media), previously prepared and sterilized, was cooled down to 45–50 °C, and 20 ml of this media were poured into 9-cm sterile glass Petri dishes previously marked suitably at the bottom surface, to a depth of approximately 4 mm. The inoculums were added to the molten agar media in the Petri dishes and the plates were swirled gently to disperse the microorganisms homogeneously. The plates were then allowed to solidify. Then wells were made with the help of a cylinder and impregnated with 50 µl of each test compounds at four different concentrations (2.5, 1.2, 0.6, and 0.3 mg/ml) and placed on the solidified surface of the media seeded with the respective microorganism. Similarly, vehicle dimethylsulfoxide (DMSO) impregnated in the well was used as the negative control. Kanamycine sulfate (10 µg/disc) was used as a positive reference standard to determine the sensitivity of each microbial species tested (Table 2). The inoculated plates were incubated at 37 °C for 24 h for bacteria strains. Antimicrobial activity was evaluated by measuring

Table 2. Minimum inhibitory concentration values of Kanamycin sulfate by Agar diffused method

Bacteria	Zone of inhibition (mm)
Staphylococcus epidermidis	30
Pseudomonas aeruginosa	15
Proteus valguris	25
Bacillus subtilis	33

the diameter of zone of inhibition against test organisms. The antibacterial activity of compounds against four bacterial strains was initially assessed by the agar-diffusion method. The compounds showed antibacterial activity against most of the tested bacteria, mainly at higher concentrations. The compounds **4a, 4b**, and **4c** exibited moderate to feeble inhibition against all test bacteria, but **4d** was best against *Staphylococcus epidermidis* (22 mm) and worst against *Pseudomonas aeruginosa* (16 mm) at the greatest concentration (2.5 mg/ml). The activities decreased with decrease in concentration. All the synthesized compounds were found to possess activity at maximum concentration. The results indicated that **4d** was more effective than the rest of the compounds. This may be due to the fact that the **4d** had more antibacterial constituents, which were less or absent in the rest of the compounds. The compounds were effective against most Gram-positive and Gram-negative bacteria tested, thereby indicating a broad spectrum of activity. However, the results revealed that the Gram-positive bacteria were in general more sensitive to this compound. In this investigation the agar disk diffusion method was employed to serve as initial antibacterial screening procedure. The diameter of zone of inhibition is a function of initial concentration (in the well), solubility, and diffusion rate of the antibacterial compounds present through the agar media and thus not a true measure of effectiveness. Many physical and chemical factors unrelated to anti-bacterial activity affect the rate of diffusion of compounds through bacteria-seeded solid media. Therefore, it is not possible to measure the antibacterial activity of a new compound in terms of another antibiotic as standard by comparing the diameter zone of inhibition produced by agar diffusion; hence in the present study no antibiotic standard was employed while the agar diffusion method was used to assess the antibacterial activity, where the compounds were diluted serially in a sequence of decreasing concentration and inoculated with test bacteria. The smallest concentration of the extract that prevented visible growth (turbidity) was called the minimum inhibitory concentration (MIC). Here, a reference broad-spectrum antibiotic, kanamycin sulfate, was employed as standard. Furthermore, some MIC values obtained for kanamycin sulfate were roughly in agreement with literature values.[32–37] Therefore, the standard antibiotic was employed not only for the comparison but also to ensure the bacteria used in the study and the experimental conditions were appropriate and acceptable. The present investigation is the first experimental demonstration of any biological activity as well as broad-spectrum antibacterial efficacy compounds. The results of the antimicrobial screening are given in Table 3. From the results obtained, it is obvious that most of the compounds showed promising activity against bacteria.

Table 3. In vitro antibacterial activity of **4a–i** (mg/ml) by agar diffusion method

		Zone of inhibition (mm)			
		Gram-positive bacteria[a]		Gram-negative bacteria[a]	
Compound	Conc. (mg/ml)	*Se*	*Bs*	*Pa*	*Pv*
4a	2.5	—	—	—	—
	1.2	—	—	—	—
	0.6	—	—	—	—
	0.3	—	—	—	—
4b	2.5	—	—	—	—
	1.2	—	—	—	—
	0.6	—	—	—	—
	0.3	—	—	—	—
4c	2.5	—	—	—	—
	1.2	—	—	—	—
	0.6	—	—	—	—
	0.3	—	—	—	—
4d	2.5	—	—	—	—
	1.2	—	—	—	—
	0.6	—	—	—	—
	0.3	—	—	—	—
4e	2.5	20	19	17	21
	1.2	17	14	—	—
	0.6	15	—	—	—
	0.3	13	—	—	—
4f	2.5	21	15	16	18
	1.2	20	13	15	15
	0.6	18	—	13	14
	0.3	17	—	—	—
4g	2.5	21	16	17	15
	1.2	19	15	14	13
	0.6	18	—	13	11
	0.3	17	—	—	10
4h	2.5	22	25	16	20
	1.2	18	15	12	18
	0.6	16	14	—	16
	0.3	14	12	—	15
4i	2.5	—	—	—	—
	1.2	—	—	—	—
	0.6	—	—	—	—
	0.3	—	—	—	—

[a]The screening organisms. Gram-positive bacteria: *Staphylococcus epidermidis* (*Se*), *Bacillus subtilis* (*Bs*). Gram-negative bacteria: *Pseudomonas aeruginosa* (*Pa*), *Proteus valguris* (*Pv*).

EXPERIMENTAL

Melting points were recorded on an Electrothermal type 9100 melting-point apparatus. The IR spectra were obtained on a 4300 Shimadzu spectrometer as pallets on KBr discs. The ^{1}H NMR (400 MHz) spectra were recorded on a Bruker Advace II 400 spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard (chemical shifts in delta, ppm).

Data are given in the following order: δ value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad), number of protons, and coupling constants *J* (given in hertz). The mass spectra were scanned on a Varian Mat CH-7 at 70 eV. All of the chemicals were purchased from Sigma, Fluka, Hi-media, and Merck. The reaction were monitored by thin-layer chromatography (TLC) using glass plates coated with silica gel-G containing 13% calcium sulfate as binder.

General Procedure for Synthesis of Pyrazole Derivatives of 4

To a mixture of **3** (3 g, 0.008 mol) in tetrahydrofuran (30 mL) and *N*-methylmorpholine (1.59 g, 0.016 mol) was slowly added *i*-butychloroformate (1.6 g, 0.012 mmol) in tetrahydrofuran (10 mL) at −10 to −20 °C, and the mixture was stirred at the same temperature for 20 min. Then a solution of respective amines (1.2 mol eq.) in tetrahydrofuran (30 mL) was added over a period of 30–45 min, and the solution was stirred for 1 hr at −10 to −20 °C. After completion of the reaction from TLC (mobile phase 2:8 methanol and dichloromethane) solvent was distilled up to get a residue, which was taken in water (100 ml), and the product was extracted with dichloromethane. The organic layer was dried over sodium sulfate, and the solvent was distilled out under vacuum to get a residue, which was crystallized with di-isopropyl ether to get solid **4a–i**.

[5-(4-Chloro-phenyl)-1-(2,4-dichloro-phenyl)-4-methyl-1H-pyrazole-3-yl]-(octahydro-isoindol-2-yl)-methanone (4a)

Anal. calcd. for $C_{25}H_{24}Cl_3N_3O$: C, 61.42; H, 4.95; N, 8.60. Found: C, 61.19; H, 4.80; N, 8.45. IR (KBr, ν_{max}, cm^{-1}): 1624 (C=O); ^{1}H NMR (400 MHz, δ, ppm, $CDCl_3$): 1.25–1.53 (m, 8H, $4 \times CH_2$ of cyclohexane ring of indolene), 2.13–2.20 (m, 5H, -CH_3& C_8-H, C_{13}-H of indolene), 3.48–3.80 (m, 4H, $2 \times CH_2$ of indolene), 6.99 (d, 2H, $J = 8.32$ Hz, $ArC_{5''}$-H & $ArC_{6''}$-H), 7.10 (d, 1H, $J = 8.44$ Hz, $ArC_{3''}$-H), 7.15–7.21(m, 3H, $ArC_{6'}$-H, $ArC_{3'}$-H & $ArC_{5'}$-H), 7.34–7.35 (d, 1H, $J = 1.88$ Hz, $ArC_{2'}$-H); ^{13}C NMR (100 MHz, δ, ppm, $CDCl_3$): 9.50 (C-7), 22.56, 22.87, 25.73, 25.78, 35.56, 37.83, 50.03, 53.08, 118.60 (carbon of indolene), 117.47 (C-6″), 127.55 (C-2″), 127.79 (C-5″), 128.85 (C-2′ & C-6′), 130.27 (C-3″), 130.56 (C-4″), 130.76 (C-3′ & C-5′), 133.03 (C-4′), 134.66 (C-1′), 135.56 (C-1″), 136.15 (C-5), 141.62 (C-3), 147.17 (C-4), 163.38 (C-6); MS (*m*/*z*): $M + 1]^+$ 488.17.

SUPPORTING INFORMATION

Experimental details, spectral and analytical data of synthesized compounds, and copies of IR, ^{1}H NMR, ^{13}C NMR, and mass spectra of compounds **2**, **3**, and **4a–j** are given in the supplementary content. This material can be found via the Supplementary Content section of this article's Web page.

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