Efficient Regiocontrolled Synthesis and Antimicrobial Activity of Pyrazoles

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Received 18 December 2006; revised 1 April 2007

Abstract: A series of 1,5-diphenyl-1*H*-pyrazol-3-amines, 3-ethoxy-5-phenyl-1*H*-pyrazole, 5-ethoxy-1,3-diphenyl-1*H*-pyrazole and 3-ethoxy-1,5-diphenyl-1*H*-pyrazole were efficiently prepared from the regiocontrolled cyclization of α-oxoketene *O*,*N*-acetals and/or β-oxo thioxoesters with hydrazine derivatives using montmorillonite K-10 as solid support with ultrasound. The antimicrobial activity of the heterocyclic compounds was evaluated by direct bioautography test.

Key words: pyrazoles, solid support K-10, regioselective synthesis, bioautography

Infections caused by microorganisms pose a serious challenge to the scientific community and the necessity for effective therapy has led to research in novel biologically active agents. The pyrazole nucleus, among its numerous pharmacological properties,[1] also possesses antimicrobial activity[2] and, thus, is an ideal candidate. The synthesis of 1-arylpyrazoles usually involves the cyclocondensation of 1,3-dicarbonyl compounds and their equivalent 1,3-dielectrophilic precursors such as β-dialkylamino-substituted chloro ketones with arylhydrazines. However, these methods afford regioisomeric mixtures of pyrazoles.[3,4] In recent years, some success has been achieved in the regioselective synthesis of 1-arylpyrazoles, however these methods require severe reaction conditions[5–8] and specific bases.[9]

Our research group has been studying the synthesis of polyfunctionalized heterocyclic systems utilizing reactions on the solid support montmorillonite K-10.[10–12] This study presents the synthesis of a series of pyrazole compounds using montmorillonite K-10 as a solid support under solvent-free conditions. These heterocyclic systems are similar to 5-(chloromethyl)-1*H*-pyrazole **1** and 5(3)-amino-substituted 3(5)-phenyl-1*H*-pyrazoles **2a**–**g** (Figure 1) which we have previously prepared.[12,13]

The heterocyclic 3-amino-substituted 1,5-diphenylpyrazoles **5a**–**e** (Scheme 1), 3-ethoxy-5-phenyl-1*H*-pyrazole (**6**) (Scheme 2) and 5-ethoxy-1,3-diphenyl-1*H*-pyrazole (**7**) (Scheme 3) were synthesized and, together with compounds **1** and **2a**–**g**, their antibacterial and antifungal activities were tested.

SYNTHESIS 2007, No. 16, pp 2485–2490
Advanced online publication: 12.07.2007
DOI: 10.1055/s-2007-983771; Art ID: M07306SS

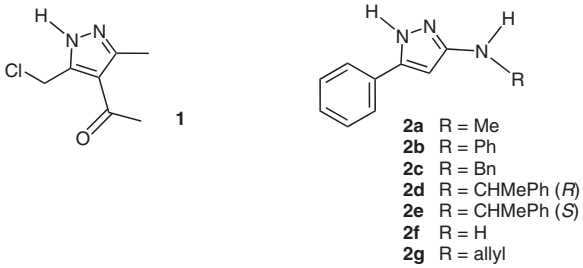
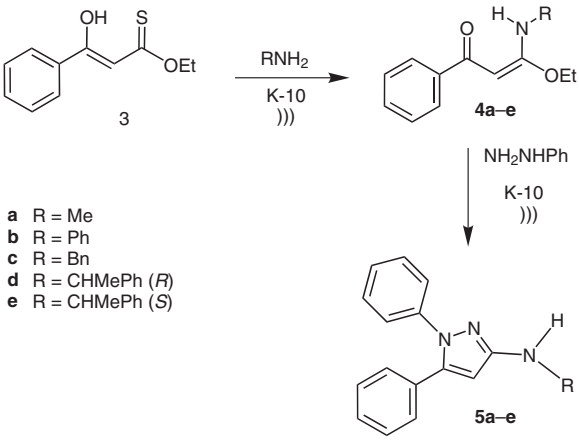


Figure 1 Structures of the heterocyclic compounds **1** and **2a**–**g**

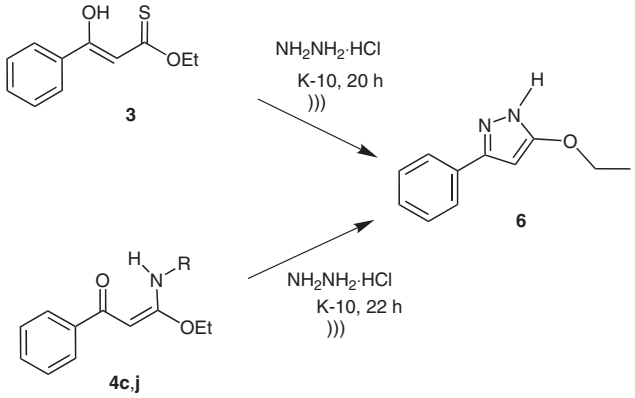
A series of 3-amino-substituted 1,5-diphenylpyrazoles **5a**–**e** was synthesized regioselectively from the cyclization reaction of α-oxoketene *O*,*N*-acetals **4a**–**e** with phenylhydrazine on montmorillonite K-10, as solid support under ultrasound (Scheme 1). The precursors **4a**–**e** are polarized ketenes representing a new class of versatile functionalized enamine carbonylic compound[14] and were prepared by reported procedures.[13]



Scheme 1 Synthesis of the 3-amino-substituted 1,5-diphenylpyrazoles **5a**–**e**

The structure of each product was identified from spectroscopic data. Due to the high selectivity observed in the formation of the pyrazole rings **5a**–**e** in heterogeneous media, we decided to investigate the reactivity of the α-oxoketene *O*,*N*-substituted acetal **4c** (R = Bn) and unsubstituted **4f** (R = H) with hydrazine and phenylhydrazine hydrochloride using montmorillonite K-10 and ultrasound sonication. The α-oxoketene *O*,*N*-acetals **4c**,**f** were reacted with hydrazine hydrochloride under solid support and ultrasound sonication to afford 3(5)-ethoxy-5(3)-phenyl-

1*H*-pyrazole **6** as shown in Scheme 2. The unexpected loss of the amino group leading to the formation of heterocyclic compound **6** is probably due to acidic conditions from the presence of the hydrazine hydrochloride, making this group a better leaving group that the ethoxy group. When the same starting materials **4c** and **4f** were used in the reaction with hydrazine hydrate in its free form, the aminopyrazoles **2c**,**g** were produced.



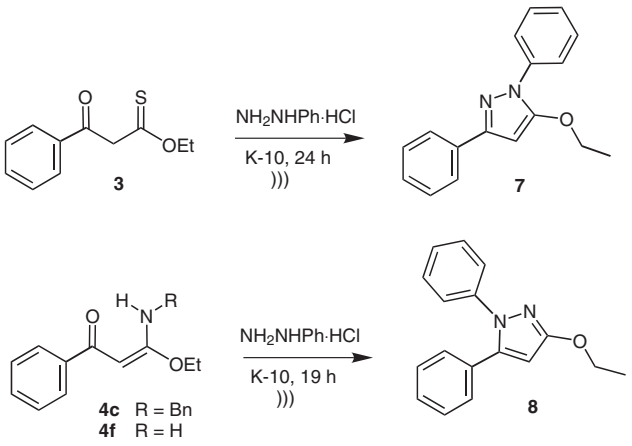
Scheme 2 Synthesis of the 3(5)-ethoxy-5(3)-phenyl-1*H*-pyrazole **6**

Due to loss of the amino group in the formation of the pyrazole **6**, we decided to repeat the cyclization reaction using the α-oxo thioxoester **3**, instead, as starting material to evaluate its reactivity with the reaction conditions used (Scheme 2). The same 1*H*-pyrazole **6** was obtained. We believed that the keto form of the compound **3** may be present in the reaction conditions used, so that through this tautomeric form the formation of pyrazole **6** is possible. The structure of the compound **6** was identified from spectroscopic data.

Continuing the study of the reactivity of the α-oxo thioxoester **3** and of the *O*,*N*-acetals **4c**,**f**, we performed the reaction of these compounds with phenylhydrazine hydrochloride using solid support and ultrasound to generate selectively the regioisomers 5-ethoxy-1,3-diphenyl-1*H*-pyrazole (**7**) and 3-ethoxy-1,5-diphenyl-1*H*-pyrazole (**8**) (Scheme 3). Compound **7** is obtained by probable initial attack of the more nucleophilic nitrogen of phenylhydrazine hydrochloride at the carbonylic carbon of β-oxo thioxoester **3**, with loss of water followed by cyclization. The formation of compound **8** is considered to proceed by initial attack of the more nucleophilic nitrogen of phenylhydrazine hydrochloride at Cβ of *O*,*N*-acetals **4c**,**f**, with loss of the amino group followed by intramolecular cyclization.

The structures of the regioisomers **7** and **8** were unambiguously established by ^{1}H and ^{13}C NMR spectra. The previously reported synthesis[15] of regioisomeric compounds similar to **7** and **8** requires specific reaction conditions such as elevated temperature and basic media.

X-ray crystallographic studies on compounds **5a**, **5c**, and **7** were undertaken to confirm the regiochemistry of the products. Figure 2 shows the molecular structures of compounds **5a** and **5c** and Figure 3 shows the two crystallographically independent molecules, labeled I and II, found in the asymmetric unit of compound **7**. All compounds contain a central pyrazole ring with three substituents, an *N*-phenyl group, a *C*-phenyl group and a secondary amine (**5a** and **5c**) or ethyl ether moiety (**7**). The X-ray study confirms that in compounds **5a** and **5c**, the *C*-phenyl group is in the 5-position, adjacent to the *N*-phenyl group, and the secondary amine is in the 3-position, while in compound **7**, the *C*-phenyl is in the 3-position and the ethyl ether is in the 5-position. The regiochemistry differences support the different mechanism of attack and cyclization. In the structures, the pyrazole ring system shows a delocalized double bond system that is almost exactly planar (root-mean-square deviations less than 0.005 Å). An analysis of the bond lengths within the pyrazole ring show that they are consistent with average bonds lengths found in the literature for *N*-phenylpyrazole compounds (42 observations) obtained from the Cambridge Structural Database[16] (Table 1).[17]



Scheme 3 Synthesis of the 5-ethoxy-1,3-diphenyl-1*H*-pyrazole (**7**) and 3-ethoxy-1,5-diphenyl-1*H*-pyrazole (**8**)

The antimicrobial activity of 5-(chloromethyl)pyrazole **1**, 3-amino-substituted 5-phenyl-1*H*-pyrazoles **2a**–**g**, 3-amino-substituted 1,5-diphenyl-1*H*-pyrazoles **5a**–**e**, 3-ethoxy-5-phenyl-1*H*-pyrazole (**6**) and 5-ethoxy-1,3-diphenyl-1*H*-pyrazole (**7**) were evaluated by direct bioautography[18] using a TLC bioassay against microorganisms strains. The collection of eleven microorganisms used included three Gram-positive bacteria: *Staphylococcus aureus* (Sa), *Staphylococcus epidermidis* (Se), *Bacillus subtilis* (Bs); four Gram-negative bacteria: *Eschericchia coli* (Ec), *Salmonella setubal* (Ss), *Pseudomonas aeruginosa* (Pa), *Klebsiella pneumoniae* (Kp); four fungi: *Candida albicans* (Ca), *Saccharomyces cerevisiae* (Sc), *Cryptococcus neoformans* (Cn), and *Candida dubliniensis* (Cd- isolated clinical). For the antimicrobial analysis, initially we compared compounds **1** and **2d**, which possess different patterns of substitution. Among the series of the aminopyrazoles **2a**–**g**, we chose the heterocycle **2d**, that has an asymmetrical center [(*R*)-CHMePh].

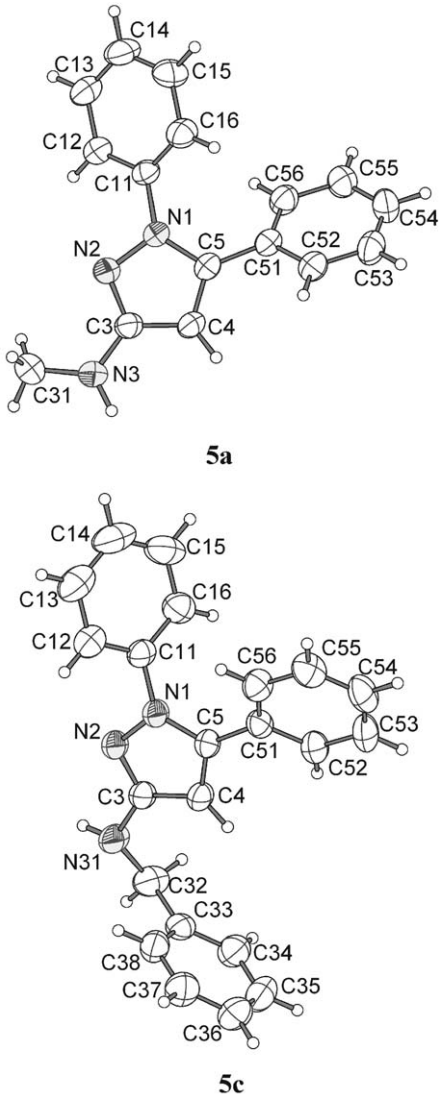


Figure 2 X-ray crystal structure of 3-(methylamino)-1,5-diphenyl-1*H*-pyrazole (**5a**) and 3-(benzylamino)-1,5-diphenyl-1*H*-pyrazole (**5c**)

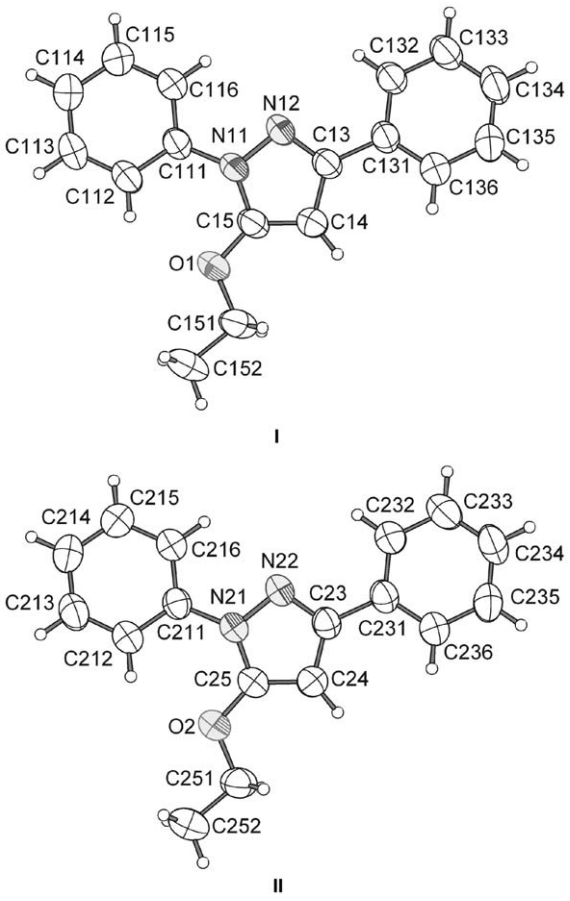


Figure 3 X-ray crystal structure of the two crystallographically independent molecules of 5-ethoxy-1,3-diphenyl-1*H*-pyrazole (**7**)

The bioassay of compound **1** shows that it presents concentration dependent activity against all tested microorganisms. Good antifungal activity is observed against all analyzed fungi, with strong inhibition against *C. dublinensis* (1.0 μg) compared to the control. Compound **2d** exhibits promising antibacterial activity against all Gram-positive and Gram-negative bacteria (Table 2). It is possible to detect the same antibacterial tendency shown by the heterocycle **2d** in the remaining compounds of the series of the 5-amino-substituted pyrazoles [R = Me, Ph, Bn, (*S*)-CHMePh, H, allyl] (Table 1). In particular, compound **2e** exhibits very good antibacterial potential (0.15 μg) against all strains of tested bacteria, however, it is inactive against all the analyzed fungi in the same way as compound **2a**. The excellent results of the bacterial inhibition demonstrated by **2e** compared that of its isomer **2d**, and with the other analyzed aminopyrazoles, suggests that the stereochemistry [(*S*)-CHMePh] present in structure of **2e** plays an important role.

Based on the promising bioactive behavior presented by the series of the aminopyrazoles **2a**–**g**, we decided to investigate the structure–activity relationship of their 3-amino-substituted 1,5-diphenylpyrazoles **5a**–**e** analogues, as well as of the heterocycles 3-ethoxy-5-phenyl-1*H*-pyrazole (**6**) (Scheme 2) and 5-ethoxy-1,3-diphenyl-1*H*-pyrazole (**7**) (Scheme 3). The bioassay of compounds **5a**–**e** shows that these heterocycles are inactive against all tested microorganisms. This finding clearly indicates that the presence of the phenyl substituent in the N1 position of the ring is responsible for the inactivity of these compounds. An identical result to those described above was observed with the ethoxypyrazole **7**, which also shows no antimicrobial activity against all the analyzed microorganisms.

The phenyl group in the N1 position continues to inhibit biological activity despite the different substituents on the pyrazole ring. On the other hand, the heterocyclic compound **6** demonstrates moderate antibacterial activity against the tested bacteria (Table 3), when compared to the group control and the series **2a**–**g**, except for the bacterium Gram-negative *K. pneumoniae* for which was not active, but, similar to **2a**, **2e**, and **5a**–**e**, it is inactive against fungi. The fact that ethoxypyrazole **6** presents bacterial inhibition against all Gram-positive bacteria and

Table 1 Bond Distances within the Pyrazole Ring for **5a**, **5c**, and **7**[a]

Compound	Bond lengths (Å)				
	N1–N2	N1–C5	N2=C3	C3–C4	C4=C5
5a	1.3887(14)	1.3698(16)	1.3337(15)	1.4075(19)	1.3634(19)
5c	1.3787(13)	1.3596(14)	1.3282(14)	1.4061(15)	1.3726(16)
7(I)	1.3887(14)	1.3666(19)	1.3243(20)	1.4043(19)	1.3595(19)
7(II)	1.3766(15)	1.3628(20)	1.3255(19)	1.4064(21)	1.3651(19)
average, *N*-phenylpyrazoles	1.371(13)	1.363(12)	1.330(9)	1.400(14)	1.366(11)
average, all compounds[17]	1.366(19)	1.336(17)	1.279(8)	1.513(14)	1.299(27

[a] With comparisons to the averages found for *N*-phenylpyrazole rings (42 observations in the C.S.D.) and for N–N, N–C, N=C, C–C, and C=C bonds for all compounds.

Table 2 Bioautography Assay Results (μg) of **1** and **2a**–**g**

Microorganisms		**1**	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	Control[a]
Staphylococcus aureus	ATCC 6538p	50	0.75	0.75	0.5	6.25	0.15	3.12	0.5	0.7
Staphylococcus epidermidis	ATCC 12228	100	3.12	0.15	0.75	1.0	0.15	3.12	25	0.7
Bacillus subtilis	ATCC 6633	25	1.56	0.5	3.12	0.5	0.15	6.25	12.5	0.8
Eschericchia coli	ATCC 25792	100	3.12	0.15	3.12	1.0	0.15	3.12	0.5	0.5
Salmonella setubal	ATCC 19796	50	1.0	0.15	1.0	0.15	0.15	3.12	12.5	0.7
Klebsiella pneumoniae	ATCC 10031	50	3.12	0.15	0.15	0.5	0.15	6.25	12.5	0.5
Pseudomonas aeruginosa	ATCC 27853	100	1.0	0.75	1.56	3.12	0.15	6.25	12.5	0.6
Saccharomyces cerevisiae	ATCC 2601	6.25	na	na	na	na	na	na	na	3.24
Candida albicans	ATCC 10231	6.25	na	na	na	na	na	na	100	2.43
Cryptococcus neoformans	ATCC 28952	3.12	na	0.75	25	na	na	100	na	2.43
Candida dubliniensis	isolated clinical SM-26	1.0	na	25	6.25	25	na	na	na	4.05

[a] Standard antibiotic chloramphenicol (bacteria) and Nistatine (fungi) (μg); na = not active.

against three Gram-negative bacteria, is due to the lack of the phenyl substituent in the position N1 of the pyrazole ring, similar the aminopyrazoles **2a**–**g**, which are active against all tested bacteria.

In summary, the reactions using montmorillonite K-10 support and ultrasound provides a highly selective method for the synthesis of 1,3- and 1,5-diphenylpyrazoles. The results of the *in vitro* biological evaluation against various microorganisms indicates that 5-(chloromethyl)pyrazole **1** demonstrates good antifungal behavior and the series 5(3)-amino-substituted 3(5)-phenyl-1*H*-pyrazoles **2a**–**g** presents strong antibacterial behavior. Furthermore, the structure–activity-relationship study comparing the results of the bioactive aminopyrazoles **2a**–**g**, and ethoxypyrazole **6**, with the bioinactive series 3-amino-1,5-diphenylpyrazoles **5a**–**e** and 5-ethoxy-1,3-diphenyl-1*H*-pyrazole (**7**) shows that the presence of the amino group in the ring enhances the antibacterial activity against the tested bacteria while the presence of the phenyl in the N1 position substituent on the pyrazole ring inhibits activity against all of the employed microorganisms.

Melting points were determined with a Microquímica APF-301 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX 200 and 400 spectrometer in $CDCl_3$/TMS. Crystallographic measurements were made on a Bruker X8 Kappa Apex II CCD area detector equipped automatic diffractometer with MoKα radiation (λ = 0.71073 Å). Structures were solved by direct methods (SHELXS-97) and additional atoms were located in the difference Fourier map and refined on F^2 (SHELXL-97). An ultrasound bath (water), Thornton 50–60 Hz, 110/220 V, 1.0 amps was used. The temperature of the water bath never exceeded 35 °C.

Table 3 Bioautography Assay Results (μg) of **6**

Microorganisms		**6**	Control[a]
Staphylococcus aureus	ATCC 6538p	50	0.7
Staphylococcus epidermidis	ATCC 12228	3.12	0.7
Bacillus subtilis	ATCC 6633	12.5	0.8
Eschericchia coli	ATCC 25792	12.5	0.5
Salmonella setubal	ATCC 19796	50	0.7
Klebsiella pneumoniae	ATCC 10031	na	0.5
Pseudomonas aeruginosa	ATCC 27853	25	0.6
Saccharomyces cerevisiae	ATCC 2601	na	3.24
Candida albicans	ATCC 10231	na	2.43
Cryptococcus neoformans	ATCC 28952	na	2.43
Candida dubliniensis	isolated clinical SM-26	na	4.05

[a] Standard antibiotic chloramphenicol (bacteria) and Nistatine (fungi) (μg); na = not active.

3-Amino-Substituted 1,5-Diphenylpyrazoles 5a–e; General Procedure

Phenylhydrazine (10 mmol) was added to the appropriate α-oxoketene *O*,*N*-acetals **4a**–**e** (5 mmol) dispersed on montmorillonite K-10, the mixture was placed in ultrasound bath for 19 h. The product was extracted by washing the montmorillonite K-10 with CH_2Cl_2; the CH_2Cl_2 soln was dried ($MgSO_4$), filtered, and the solvent was removed in vacuo to yield the crude product. All the compounds were purified by column chromatography (silica gel, Aldrich 70–230 mesh, EtOAc–hexane) to give analytically pure pyrazoles.

3-(Methylamino)-1,5-diphenyl-1*H*-pyrazole (5a)

Chromatography: 30% EtOAc–hexane; solid; yield: 39%; mp 107–109 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.19–7.28 (m, 10 H), 5.92 (s, 1 H), 3.94 (br, 1 H), 2.93 (s, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.62, 144.14, 139.96, 130.69, 128.72, 128.58, 128.31, 128.18, 126.48, 124.76, 94.56, 31.57.

1,5-Diphenyl-3-(phenylamino)-1*H*-pyrazole (5b)

Chromatography: 25% EtOAc–hexane; oil; yield: 43%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.19–7.32 (m, 15 H), 6.31 (br, 1 H), 6.26 (s, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.97, 143.60, 142.81, 139.97, 130.59, 130.59, 129.17, 128.74, 128.68, 128.40, 128.30, 126.68, 124.77, 120.10, 116.13, 97.23.

3-(Benzylamino)-1,5-diphenyl-1*H*-pyrazole (5c)

Chromatography: 10% EtOAc–hexane; solid; yield: 19%; mp 114–116 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.14–7.42 (m, 15 H), 5.83 (s, 1 H), 4.42 (s, 2 H), 3.87 (br, 1 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 157.19, 143.84, 140.14, 139.81, 130.85, 128.61, 128.56, 128.42, 128.25, 128.05, 127.54, 127.04, 126.25, 124.62, 94.73, 48.96.

1,5-Diphenyl-3-{[(*R*)-1-phenylethyl]amino}-1*H*-pyrazole (5d) and 1,5-Diphenyl-3-{[(*S*)-1-phenylethyl]amino}-1,5-diphenyl-1*H*-pyrazole (5e)

Compound 5d

Chromatography: 10% EtOAc–hexane; oil; yield: 10%.

Compound 5e

Chromatography: 25% EtOAc–hexane; oil; yield: 21%.

Compounds 5d and 5e

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.05–7.33 (m, 15 H), 5.55 (s, 1 H), 4. 58 (br, 1 H), 4.46 (q, *J* = 6.8 Hz, 1 H), 1.45 (d, *J* = 6.8 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.37, 145.21, 143.74, 139.80, 130.62, 128.62, 128.54, 128.45, 128.21, 128.10, 126.87, 126.36, 126.04, 124.56, 94.38, 54.57, 24.35.

3-Ethoxy-5-phenyl-1*H*-pyrazole (6)

$PhNHNH_2$·HCl (10 mmol) was added to the α-oxoketene *O*,*N*-acetal **4c** or **4f** or to the α-oxo thioxoester **3** (5 mmol) dispersed on montmorillonite K-10, the mixture was placed in ultrasound bath for 20 h or 22 h. The product was extracted by washing the montmorillonite K-10 with CH_2Cl_2; the CH_2Cl_2 soln was dried ($MgSO_4$), filtered, and the solvent was removed in vacuo to yield the crude product. The compound was purified by column chromatography (silica gel, Aldrich 70–230 mesh, 15% EtOAc–petroleum ether) to give analytically pure pyrazole as a solid; yield: 43% (from **4c**), 59% (from **4f**), and 47% (from **3**); mp 127–129 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.60 (m, 5 H), 5.93 (s, 1 H), 4.22 (q, *J* = 6.8 Hz, 2 H), 1.37 (t, *J* = 6.8 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.96, 144.95, 129.98, 128.87, 128.44, 125.36, 87.93, 65.00, 14.84.

5-Ethoxy-1,3-diphenyl-1*H*-pyrazole (7)

$PhNHNH_2$·HCl (10 mmol) was added to the α-oxo thioxoester **3** (5 mmol) dispersed on montmorillonite K-10 and the mixture was placed in ultrasound bath for 24 h. The product was extracted by washing the montmorillonite K-10 with CH_2Cl_2; the CH_2Cl_2 soln was dried ($MgSO_4$), filtered, and the solvent was removed in vacuo to yield the crude product. The compound was purified by column chromatography (silica gel, Aldrich 70–230 mesh, 15% EtOAc–hexane) to give analytically pure **7** as a solid; yield: 52%; mp 67–69 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.23–7.86 (m, 10 H), 5.97 (s, 1 H), 4.21 (q, *J* = 7.0, 2 H), 1.46 (t, *J* = 7.0, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.24, 150.43, 138.77, 133.32, 128.73, 128.47, 127.97, 126.04, 125.49, 122.02, 83.74, 68.02, 14.56.

3-Ethoxy-1,5-diphenyl-1*H*-pyrazole (8)

$PhNHNH_2$·HCl (10 mmol) was added to the α-oxoketene *O*,*N*-acetal **4c** or **4f** (5 mmol) dispersed on montmorillonite K-10, the mixture was placed in ultrasound bath for 19 h. The product was extracted by washing the montmorillonite K-10 with CH_2Cl_2; the CH_2Cl_2 soln was dried ($MgSO_4$), filtered, and the solvent was removed in vacuo to yield the crude product. The compound was purified by column chromatography (silica gel, Aldrich 70–230 mesh, 10% EtOAc–hexane) to give analytically pure **8** as an oil; yield: 38% (from **4c**) and 63% (from **4f**).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.19–7.31 (m, 10 H), 5.95 (s, 1 H), 4.31 (q, *J* = 7.0 Hz, 2 H), 1.43 (t, *J* = 7.0 Hz, 3 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.53, 144.11, 140.11, 130.77, 128.74, 128.69, 128.37, 128.27, 126.61, 124.93, 93.69, 64.71, 14.88.

Crystallographic Measurements[19]

Hydrogen atoms were found in the difference Fourier map but refined in idealized geometric positions (C_{phenyl}–H = 0.93; $C_{methylene}$–H = 0.97; N–H = 0.89 Å). The space groups were determined by systematic absences and overall intensity statistics for compounds **5a** and **5c**. For compound **7**, the space group could not be easily determined. Crystalline metric and Laue symmetry suggested a monoclinic *C* space group without any additional translational symmetry. Intensity statistics suggested a noncentrosymmetric space group. Direct methods solutions (SHELXS-97) were tried unsuccessfully in *C*2, *Cm* and *C*2/*m* without any satisfactory result; the space groups possessed inappropriate additional symmetry causing the found molecules to fuse together. An acceptable soln was found in the primitive space group, *P*1, at half the volume of the *C* centered one. ROTAX[20] found a twofold axis about [–1 1 0]. Applying the twin law [0 –1 0 –1 0 0 0 0 –1] allowed the satisfactory completion of the refinement of the structure.

Crystal data for **5a**: $C_{16}H_{15}N_3$, M = 249.31, orthorhombic, space group *Pbca* (No. 61), a = 8.5449(5) Å, b = 12.2609(9) Å, c = 26.0612(17) Å, V = 2730.4(3) Å^3, T = 294(2) K, Z = 8, D_c = 1.213 g/cm^{-3}, μ = 0.074 mm^{-1}, 3.01 < θ < 29.61°, F(000) = 1056; 15574 reflections measured, 3785 unique (R_{int} = 0.0521). The final wR_2 = 0.1175 (all data), R_1 [I > $\sigma(I)$] = 0.0429, GoF = 0.905. CCDC No. 611960.

Crystal data for **5c**: $C_{22}H_{19}N_3$, M = 325.40, monoclinic, space group $P2_1/c$ (No. 14), a = 5.6704(2) Å, b = 19.0708(8) Å, c = 16.5995(7) Å, β = 98.942(2)°, V = 1773.24(12) Å^3, T = 294(2) K, Z = 4, D_c = 1.219 g/cm^{-3}, μ = 0.073 mm^{-1}, 3.44 < θ < 29.74°, F(000) = 688; 20995 reflections measured, 5020 unique (R_{int} = 0.0299). The final wR_2 = 0.1406 (all data), R_1 [I > $\sigma(I)$] = 0.0444, GoF = 1.013. CCDC No. 611961.

Crystal data for **7**: $C_{17}H_{16}N_2O$, M = 264.32, triclinic, space group *P*1 (No. 2), a = 9.7703(2) Å, b = 9.7746(2) Å, c = 15.3970(3) Å, α = 97.0210(10)°, β = 97.0020(10)°, γ = 93.0100(10), V = 1445.06(5) Å^3, T = 294(2) K, Z = 4, D_c = 1.215 g/cm^{-3}, μ = 0.077 mm^{-1}, 1.34 < θ < 30.07°, F(000) = 560; 63439 reflections measured, 8442 unique (R_{int} = 0.0291). The final wR_2 = 0.1314 (all data), R_1 [$I > \sigma(I)$] = 0.0425, GoF = 1.042. CCDC No. 611962.

Antimicrobial Activities

The antimicrobial activities of the compounds **1**, **2a–g**, **3a–e**, **6**, and **7** were assayed using the bioautography technique.[18] The collection of eleven microorganisms used included three Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538p), *Staphylococcus epidermidis* (ATCC 12228), and *Bacillus subtilis* (ATCC 6633); four Gram-negative bacteria: *Eschericchia coli* (ATCC 25792), *Salmonella setubal* (ATCC 19796), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 10031); four fungi: *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* (ATCC 2601), *Cryptococcus neoformans* (ATCC 28952), and *Candida dubliniensis* (isolated clinical SM-26). Standard microorganism strains were maintained at the Chemistry Department of the University of Santa Maria, RS, Brazil. For bioassay, the compounds were applied to pre-coated TLC plates in concentrations from 100 to 0.15 µg. Mueller–Hinton agar medium (MHA-Merck) was inoculated with microorganisms suspended in saline soln (10^5 CFU/mL) and distributed over TLC plates. Bacterium and yeast plates were incubated at 37 °C for 24 h and at 25 °C for 72 h, respectively. Standard antibiotic chloramphenicol and nistatine were used to control the sensitivity of the microbial test. After incubation, the plates were stained with an aqueous soln of 2,3,5-triphenyltetrazolium chloride (TTC, 1 mg/mL). The appearance of inhibition zones was used to demonstrate the lesser sample amount that inhibited microorganism growth. Samples were tested in triplicate.

Acknowledgment

We are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq -PIBIC), Brazil for fellowships to S.F.R.

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