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One-step synthesis, crystallographic studies and antimicrobial activity of new 4-diazopyrazole derivatives

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Summary — A number of new 4-diazopyrazole derivatives were prepared by the reaction of 1-*R*-3-methyl-5(R_1-substituted)benzamidopyrazoles with a sevenfold excess of nitrous acid in acetic medium. The compounds were tested for activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Listeria monocytogenes*, *Candida albicans*, *Candida tropicalis* and *Paecilomyces varioti*. The highest microbial susceptibility was shown by Gram-positive bacteria, with minimum inhibitory concentrations (MIC) in the range 0.5–12.5 μg/mL. For *S aureus* the R_1 substituents were screened utilizing the Topliss operational scheme. The 4-nitro group was found to be the best substituent. We also tested the compounds **4l,o,p**, found to be the most active in the test against *S aureus* ATCC 25923, on ten clinical *S aureus* strains, five of which were sensitive and five resistant to methicillin. The above compounds were active in the range 2–8 μg/mL against methicillin-resistant *S aureus* strains. An X-ray analysis of compounds **4i** and **4q** is reported.

4-diazopyrazole derivative / antibacterial activity / antifungal activity / structure

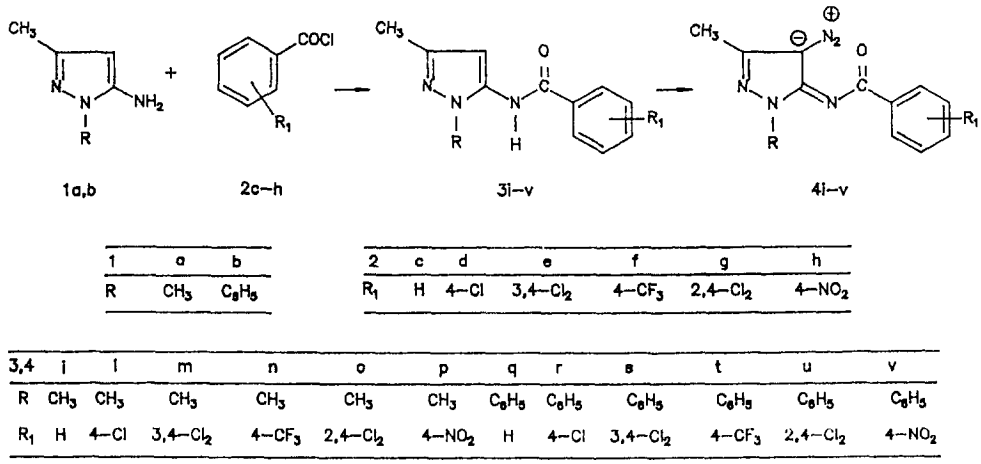
Introduction

Diazoazoles represent a class of biologically-active substances, primarily acting as antimicrobial and antineoplastic agents [1]; they are also intermediates for the synthesis of drugs and azo dyes. The bioactivity of diazoazoles is probably due to reactivity towards the nucleophylic groups of essential biomolecules such as enzymes and proteins. From a medical viewpoint, the triazene derivatives are the most important, and Dacarbazine, 5-(3,3-dimethyl-1-triazeno)-4-carboxamide-imidazole, is a well known antineoplastic drug [2].

We previously reported the reaction of 1-phenyl-3-methyl-5-(2-nitrobenzamido)pyrazole with nitrous acid in acetic medium to produce a mixture of products from which the 4-nitroso, 4-nitro, 4-chloro and 4-diazopyrazole derivatives were isolated [3]. The 4-diazo and 4-nitrosopyrazole derivatives showed growth inhibitory activity against *S aureus* in vitro. We also found that reaction of the analogues of 5-benzamidopyrazoles **3** with a sevenfold excess of nitrous acid produced the corresponding 4-diazopyrazole derivatives in 65–80% yields [4]. A similar one-step synthesis has now been applied to obtain a series of new 4-diazopyrazoles **4**, substituted in both the pyrazole and benzene nuclei, as possible antimicrobial agents (see scheme 1). In order to identify diazopyrazole derivatives with maximum antibacterical activity for *S aureus*, a variety of substituents and their positions were evaluated using the Topliss operational scheme. For two of the new compounds of this series, the molecular structure was established by X-ray analysis.

Results and discussion

The 5-benzamidopyrazoles **3**, used as starting compounds, and the 4-diazopyrazole derivatives **4** were prepared following previously reported methods [4]. The *N*-phenyl or *N*-methyl substitution of the pyrazole nucleus produces a negative charge at the diazo structure, originating on the nitrogen atom of the benzamide moiety and then delocalized over the neighbouring atoms (fig 1). All new products were characterized by microanalyses, infrared (IR) spectra and nuclear magnetic resonance (NMR) spectra. The IR spectra of the diazo compounds showed absorptions at about 2250 cm^{-1}, attributed to the diazo group; the bonds in the 1600–1620 cm^{-1} region are ascribed to



Scheme 1.

the amidic carbonyl group. The relatively low strength of the absorption arising from ν(CO) of the amide might result from an electrostatic interaction between the positive charge of the diazo group and the partial negative charge of the polarized carbonyl group. The relatively short intramolecular contacts in the molecules **4q** and **4i**, discussed in the following section, support this interpretation. This situation is also reminiscent of the dipole–dipole interaction of a hydrogen-bonded carbonyl group, which causes a lowering of the carbonyl stretching frequency. These results suggest that the zwitterionic formulation **4b** (fig 1) cannot be a unique structure for this class of 'unconventional' 4-diazopyrazoles, as previously reported by several

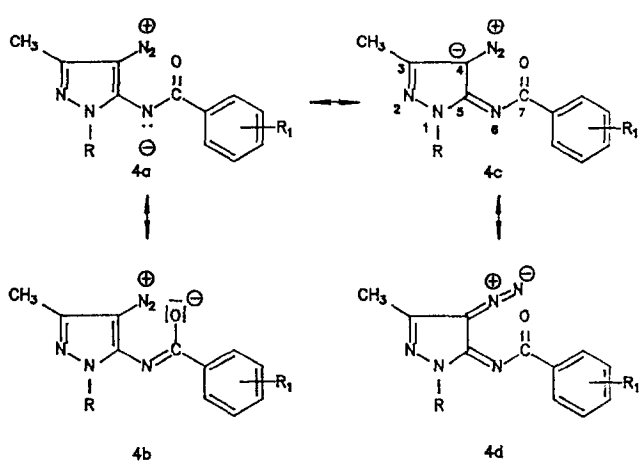


Fig 1. Resonance forms of the diazopyrazole structure.

authors [6, 7, 8]. The diazopyrazole structure should be described at least by the resonance forms **4a,b,c,d**. Furthermore **4c** appears to be the resonance form that most closely represents the structure of this class of compounds since the pyrazole C(4) atom in the 4-diazopyrazoles **4i,q** is strongly shielded. Their ^{13}C NMR spectra show resonances at 73.46 and 74.09 respectively, whereas for the amides **3i,q** the resonance occurs at 100.45 and 99.58 δ respectively, due to the lack of the 4-diazo group. Moreover, X-ray analysis of **4i,q** showed that the C(1)–N(3) bond is shorter than the N(3)–C(6) (see tables IV and V).

X-ray Study

Ortep views of the **4i** and **4q** molecules are shown in figures 2 and 3. Crystal data and some experimental details are summarized in table I. The atomic coordinates are reported in tables II and III, and selected bond lengths and angles in tables IV and V. The molecules of the methyl- (**4i**) and phenyl- (**4q**) substituted pyrazoles differ slightly in the lengths of the N(1)–C(1) bonds, which are shorter in **4i** (1.343(5) Å) than in **4q** (1.375(3) Å). The latter value is comparable to that found in the two conformers of 4-chloro-3-methyl-5-[2-(methylcabonyl)phenyl]-1-phenyl-1*H*-pyrazole (1.381(4) and 1.382(5) Å) [9]. The shortening of the N(1)–C(1) bond distance results in a decrease of the C(1)–N(1)–C(5) angle from 129.2(2)° (**4q**) to 125.4(4)° (**4i**), and a reduction of the intramolecular N(3)···C(5) contact from 2.966(3) to 2.850(6) Å. In contrast, the N(1)–C(5) bond distance, which involves a methyl group in **4i** and a phenyl group in **4q**, remains unchanged at 1.435(6) Å (**4i**) and 1.431(4) Å

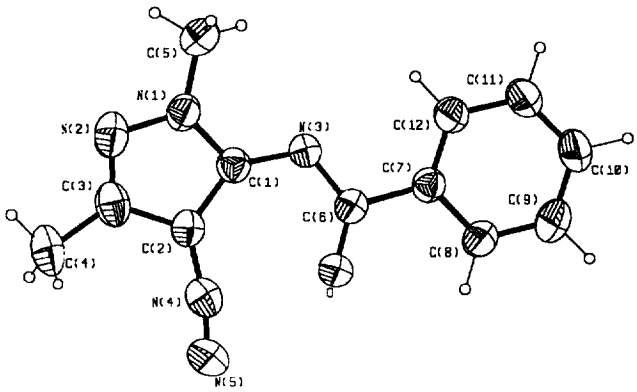


Fig 2. Molecular conformation of compound **4i** with numbering scheme. Anisotropic thermal parameters are at the 50% probability level. H atoms are shown as spheres of arbitrary radius.

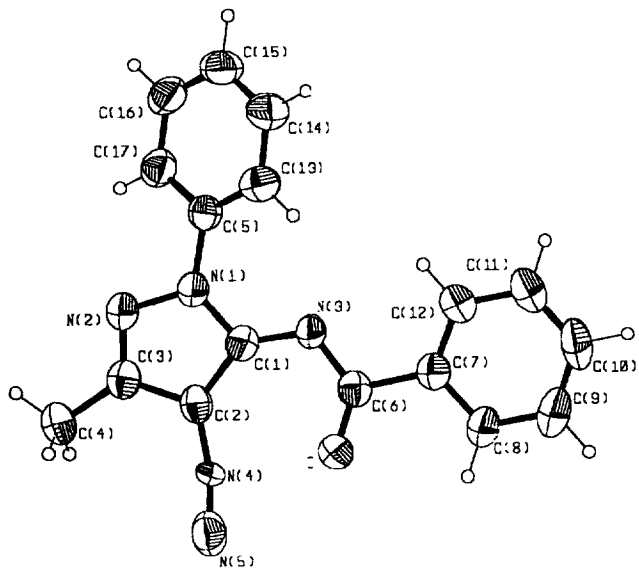


Fig 3. Molecular conformation of compound **4q** with numbering scheme. Anisotropic thermal parameters are at the 50% probability level. H atoms are shown as spheres of arbitrary radius.

Table I. Experimental data for the crystallographic analyses.

Compound	***4i***	***4q***
Formula	$C_{12}H_{11}N_5O$	$C_{17}H_{13}N_5O$
Mol wt	241.25	303.32
Crystal size (mm)	0.40 × 0.28 × 0.22	0.40 × 0.42 × 0.24
Crystal system	Monoclinic	Orthorhombic
Space group	C2/c	Pbca
a (Å)	15.616(3)	11.688(3)
b (Å)	13.057(3)	12.054(3)
c (Å)	13.579(2)	21.728(3)
β (°)	117.48(4)	
V (Å^3)	2456(1)	3061(1)
Z	8	8
Dc (g cm^{-3})	1.305	1.316
F(000)	1008	1264
2θ range (°)	2–25	2–25
Radiation λ (Å)	Mo-Kα(0.71069)	Mo-Kα(0.71069)
μ (cm^{-1})	0.84	0.81
T (K)	293	293
No reflections collected	2478	3851
No observed [$I \geq 3\sigma(I)$]	1085	1859
Weighting scheme w	$[\sigma^2(Fo) + 0.016363(Fo)^2]^{-1}$	$[\sigma^2(Fo) + 0.001608(Fo)^2]^{-1}$
$R = \Sigma[\|Fo\| - \|Fc\|]/\Sigma\|Fo\|$	0.058	0.046
$R_w = \Sigma w[\|Fo\| - \|Fc\|]^2/\Sigma w\|Fo^2\|^{1/2}$	0.069	0.054
Goodness of fit	0.75	1.35

(**4q**). Both compounds present a *trans*-planar conformation of the C(1)–N(3)–C(6)–C(7) fragments, with torsion angles of –178.9(4)° in **4i** and 176.0(2)° in **4q**. The torsion angles C(5)–N(1)–C(1)–C(2) are also comparable: –178.8(4)° and –177.6(3)° in **4i** and **4q** respectively. A significant difference between the two compounds is observed for the N(4)···O intramolecular contact (2.459(5) Å in **4i**; 2.406(3) Å in **4q**) accompanied by a different bending of the C(2)–N(4)–N(5) diazogroup (171.0(6)° in **4i** and 167.5(3)° in **4q**), and slightly shorter C(2)–N(4) and N(4)–N(5) bond distances in **4q** than **4i** (tables IV and V). These differences suggest a somewhat different population of the resonance forms in the two derivatives, as indicated also by the different torsion angles C(1)–N(3)–C(6)–O (–4.5(4)° in **4q**; 1.6(6)° in **4i**. The overall conformation of the two molecules is essentially planar, except for the phenyl attached to N(1) in **4q**, which forms an angle of 27.6(1)° with the pyrazole moiety. The dihedral angles between the pyrazole rings and the C(1)–N(3)–C(6)–O–C(7) chains are 4.45(9) and 2.3(2)° in **4q** and **4i** respectively. This smaller angle in **4i** favours a better conjugation of the N(1)–C(1)–N(3) bonds and accounts for the shorter value of the N(1)–C(1) bond length mentioned. The crystal packing is different in the two compounds; in **4q** the molecules

Table II. Fractional coordinates for non-hydrogen atoms with equivalent isotropic thermal parameters ($Å^2 \times 10^3$) for compound **4i**.

Atom	*x/a*	*y/b*	*z/c*	*Ueq*
O	0.0937(2)	−0.0891(2)	0.1693(3)	87(2)
N(1)	−0.1061(2)	0.1355(3)	0.0756(3)	67(2)
N(2)	−0.2002(2)	0.1032(3)	0.0391(3)	79(2)
N(3)	0.0523(2)	0.0809(2)	0.1419(3)	59(1)
N(4)	−0.0796(2)	−0.1280(4)	0.0896(3)	86(2)
N(5)	−0.0743(3)	−0.2133(4)	0.0888(5)	88(4)
C(1)	−0.0408(3)	0.0597(3)	0.1044(3)	59(2)
C(2)	−0.1006(2)	−0.0297(3)	0.0819(3)	62(2)
C(3)	−0.1981(3)	0.0039(4)	0.0419(3)	71(2)
C(4)	−0.2846(3)	−0.0616(4)	0.0073(4)	91(3)
C(5)	−0.0851(4)	0.2429(4)	0.0799(5)	96(3)
C(6)	0.1153(3)	0.0017(3)	0.1739(3)	59(2)
C(7)	0.2188(3)	0.0324(3)	0.2170(3)	58(2)
C(8)	0.2877(3)	−0.0435(3)	0.2398(4)	70(2)
C(9)	0.3842(3)	−0.0176(4)	0.2845(4)	86(2)
C(10)	0.4128(3)	0.0822(4)	0.3088(4)	88(3)
C(11)	0.3454(3)	0.1575(4)	0.2860(4)	82(2)
C(12)	0.2484(3)	0.1330(3)	0.2396(4)	69(2)

Table III. Fractional coordinates for non-hydrogen atoms with equivalent isotropic thermal parameters ($Å^2 \times 10^3$) for compound **4q**.

Atom	*x/a*	*y/b*	*z/c*	*Ueq*
O	−0.0474(2)	0.1205(2)	0.4440(1)	60(1)
N(1)	0.2387(2)	−0.0660(2)	0.4214(1)	47(1)
N(2)	0.3319(2)	−0.0272(2)	0.4548(1)	52(1)
N(3)	0.0464(2)	−0.0263(2)	0.3992(1)	44(1)
N(4)	0.1259(2)	0.1606(2)	0.4995(1)	31(1)
N(5)	0.0989(2)	0.2322(3)	0.5272(1)	67(1)
C(1)	0.1414(2)	−0.0034(2)	0.4291(1)	40(1)
C(2)	0.1799(2)	0.0792(2)	0.4721(1)	43(1)
C(3)	0.2979(2)	0.0592(2)	0.4851(1)	47(1)
C(4)	0.3726(3)	0.1242(3)	0.5266(2)	62(1)
C(5)	0.2574(2)	−0.1596(2)	0.3822(1)	44(1)
C(6)	−0.0441(2)	0.0420(2)	0.4077(1)	42(1)
C(7)	−0.1469(2)	0.0160(2)	0.3692(1)	45(1)
C(8)	−0.2410(2)	0.0841(3)	0.3733(2)	62(1)
C(9)	0.3390(3)	0.0613(3)	0.3390(2)	78(1)
C(10)	0.3421(3)	0.0304(3)	0.3018(2)	74(1)
C(11)	−0.2488(3)	−0.0976(3)	0.2973(2)	73(1)
C(12)	−0.1509(3)	0.0749(3)	0.3307(1)	59(1)
C(13)	0.1705(3)	−0.2325(2)	0.3674(1)	56(1)
C(14)	0.1952(3)	−0.3235(3)	0.3309(2)	65(1)
C(15)	0.3040(3)	−0.3418(3)	0.3089(2)	68(1)
C(16)	0.3898(3)	−0.2689(3)	0.3244(1)	63(1)
C(17)	0.3679(2)	−0.1772(3)	0.3607(1)	53(1)

Table IV. Selected bond lengths (Å) and angles (°) for compound **4i**.

N(1)–N(2)	1.382(5)	N(1)–C(1)	1.343(5)
N(1)–C(5)	1.435(6)	N(2)–C(3)	1.297(6)
N(3)–C(1)	1.329(5)	N(3)–C(6)	1.354(5)
N(4)–N(5)	1.116(7)	N(4)–C(2)	1.317(6)
O–C(6)	1.227(5)	C(1)–C(2)	1.438(6)
C(2)–C(3)	1.429(6)	C(3)–C(4)	1.480(6)
C(6)–C(7)	1.498(5)	C(7)–C(8)	1.388(6)
C(7)–C(12)	1.380(6)	C(8)–C(9)	1.382(6)
C(9)–C(10)	1.368(7)	C(10)–C(11)	1.367(7)
C(11)–C(12)	1.382(6)		
C(1)–N(1)–C(5)	125.4(4)	N(2)–N(1)–C(5)	119.9(4)
N(2)–N(1)–C(1)	114.7(4)	N(1)–N(2)–C(3)	106.7(4)
C(1)–N(3)–C(6)	117.9(3)	N(5)–N(4)–C(2)	171.0(6)
N(1)–C(1)–N(3)	120.5(4)	N(3)–C(1)–C(2)	137.7(4)
N(1)–C(1)–C(2)	101.8(4)	N(4)–C(2)–C(1)	131.4(4)
C(1)–C(2)–C(3)	107.8(4)	N(4)–C(2)–C(3)	120.8(4)
N(2)–C(3)–C(2)	109.0(4)	C(2)–C(3)–C(4)	126.8(4)
N(2)–C(3)–C(4)	124.2(4)	N(3)–C(6)–O	125.4(4)
O–C(6)–C(7)	120.1(4)	N(3)–C(6)–C(7)	114.5(3)
C(6)–C(7)–C(12)	122.2(4)	C(6)–C(7)–C(8)	118.8(4)
C(8)–C(7)–C(12)	119.0(4)	C(7)–C(8)–C(9)	120.0(4)
C(8)–C(9)–C(10)	120.5(5)	C(9)–C(10)–C(11)	119.9(5)
C(10)–C(11)–C(12)	120.3(4)	C(7)–C(12)–C(11)	120.3(4)

Table V. Selected bond lengths (Å) and angles (°) for compound **4q**.

O–C(6)	1.232(3)	N(1)–N(2)	1.391(3)
N(1)–C(1)	1.375(3)	N(1)–C(5)	1.431(4)
N(2)–C(3)	1.295(4)	N(3)–C(1)	1.316(3)
N(3)–C(6)	1.354(3)	N(4)–N(5)	1.099(4)
N(4)–C(2)	1.309(4)	C(1)–C(2)	1.437(4)
C(2)–C(3)	1.428(4)	C(3)–C(4)	1.480(4)
C(5)–C(13)	1.381(4)	C(5)–C(17)	1.389(4)
C(6)–C(7)	1.498(4)	C(7)–C(8)	1.376(4)
C(7)–C(12)	1.378(4)	C(8)–C(9)	1.394(5)
C(9)–C(10)	1.369(6)	C(10)–C(11)	1.361(5)
C(11)–C(12)	1.383(5)	C(13)–C(14)	1.383(4)
C(14)–C(15)	1.376(5)	C(15)–C(16)	1.375(5)
C(16)–C(17)	1.382(5)		
C(1)–N(1)–C(5)	129.2(2)	N(2)–N(1)–C(5)	117.1(2)
N(2)–N(1)–C(1)	113.5(2)	N(1)–N(2)–C(3)	107.1(2)
C(1)–N(3)–C(6)	117.6(2)	N(5)–N(4)–C(2)	167.5(3)
N(1)–C(1)–N(3)	121.4(2)	N(3)–C(1)–C(2)	136.9(2)
N(1)–C(1)–C(2)	101.6(2)	N(4)–C(2)–C(1)	131.6(2)
C(1)–C(2)–C(3)	108.3(2)	N(4)–C(2)–C(3)	120.1(3)
N(2)–C(3)–C(2)	109.4(3)	C(2)–C(3)–C(4)	127.0(3)
N(2)–C(3)–C(4)	123.6(3)	N(1)–C(5)–C(17)	117.6(2)
N(1)–C(5)–C(13)	121.9(2)	C(13)–C(5)–C(17)	120.6(3)
O–C(6)–N(3)	125.4(2)	N(3)–C(6)–C(7)	115.0(2)
O–C(6)–C(7)	119.6(2)	C(6)–C(7)–C(12)	122.2(2)
C(6)–C(7)–C(8)	118.7(3)	C(8)–C(7)–C(12)	119.1(3)
C(7)–C(8)–C(9)	120.3(3)	C(8)–C(9)–C(10)	119.7(3)
C(9)–C(10)–C(11)	120.2(3)	C(10)–C(11)–C(12)	120.4(3)
C(7)–C(12)–C(11)	120.2(3)	C(5)–C(13)–C(14)	118.9(3)
C(13)–C(14)–C(15)	121.3(3)	C(14)–C(15)–C(16)	119.1(3)
C(15)–C(16)–C(17)	121.1(3)	C(5)–C(17)–C(16)	119.1(3)

are well separated, while in **4i** intermolecular interactions are present. As illustrated in figure 4, the layers of parallel molecules in **4i** form columns in the *c* axis direction, to which they are perpendicular; the molecular separation is about 3.4 Å. The crystal cohesion is completed by inter-column contacts between one of the phenyl hydrogens and a C–O oxygen of an adjacent column (C(11)···O' 3.419(7) Å, H(11)···O' 2.441(5) Å and C(11)–H(11)···O' 176(1)°) (' at 1/2–x, 1/2 + y,1/2–z).

Biological results

Compounds **4** were evaluated, by a dilution agar method, for in vitro antimicrobial activity against representative Gram-negative (*Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive (*Staphylococcus aureus* ATCC 25923; *Staphylocccus epidermidis* ATCC 12228; *Streptococcus faecalis* ATCC 29212; *Listeria monocytogenes* NCTC 7973) bacteria, yeasts (*Candida albicans* ATCC 10231; *Candida tropicalis* ATCC 13803) and a mycelial fungus (*Paecilomyces varioti* ATCC 16023). The minimum inhibitory concentrations (MIC) of the tested compounds, compared to that of Amikacin (for bacteria) or Amphotericin B (for fungi), are reported in table VI.

All 4-diazo derivatives were found to be active in the 0.5–12.5 μg/mL range against *S aureus*, *S epidermidis*, *S faecalis* and *L monocytogenes*, *S aureus* being less susceptible than the other Gram-positive bacteria.

In contrast, compounds **4** were not active against the Gram-negative bacteria *E coli* and *P aeruginosa*, even at the maximum tested concentration (50 μg/mL). The lower microbial susceptibility of Gram-negative bacteria might be related to the complex structure of their cell wall. The *N*-phenyl substituted 4-diazopyrazole derivatives were found to be less active than their *N*-methyl analogues. This may be due to a better balance of lipophilic/hydrophilic properties for the *N*-methyl derivatives, although steric effects cannot be ruled out.

In particular, in the test against *S aureus* ATCC 25923, the Topliss sequential method allowed the identification of the most effective substitution pattern for the phenyl group of the benzamide moiety, even though only small differences in antibacterial activity were observed since the substituents are fairly removed from the diazo group and do not exert strong electronic effects on the active centre. In the methyl series, the 4-nitro substituent was the most effective, whereas in the phenyl series, the 4-nitro and 2,4-Cl_2 substituents contributed nearly to the same extent to the antibacterial activity.

Considering the important part played in human pathology by multi-resisting strains of *S aureus*, we tested the compounds **4l,o,p**, found to be the most active in the test against *S aureus* ATCC 259231, on ten strains of *S aureus* of clinical origin isolated from a variety of biological samples. Five of these isolates were considered sensitive and five resistant to the methicillin antibiotic in the classic susceptibility test.

Table VI. In vitro antimicrobial activities (MIC values μg/mL or, in parenthesis, μmol/L)

	R	R_1	*Ec*	*Pa*	*Sa*	*Se*	*Sf*	*Lm*	*Ca*	*Ct*	*Pv*
4i	CH_3	H	>50	>50	4 (16)	2	0.5	1	6.2	50	>50
4l	CH_3	4-Cl	>50	>50	3.1 (11.2)	2	0.5	2	6.2	>50	>50
4m	CH_3	3,4-Cl_2	>5	>50	4 (12.8)	2	0.5	2	25	>50	25
4n	CH_3	4-CF_3	>50	>50	12.5 (40)	6.2	2	3.1	>50	50	>50
4o	CH_3	2,4-Cl_2	>50	>50	3.1 (9)	2	1	2	12.5	25	50
4p	CH_3	4-NO_2	>50	>50	2 (6.9)	2	0.5	2	6.2	25	25
4q	C_6H_5	H	>50	>50	8 (26)	3.1	3.1	2	25	>50	>50
4r	C_6H_5	4-Cl	>50	>50	7.5 (22)	3.1	1	2	>50	>50	>50
4s	C_6H_5	3,4-Cl_2	>50	>50	10 (26.8)	2	2	2	>50	>50	>50
4t	C_6H_5	4-CF_3	>50	>50	12.5 (33.6)	6.2	6.2	6.2	>50	>50	>50
4u	C_6H_5	2.4-Cl_2	>50	>50	6.2 (16.6)	6.2	3.1	3.1	>50	12.5	50
4v	C_6H_5	4-NO_2	>50	>50	6.2 (18)	12.5	6.2	12.5	>50	>50	>50
AMK			10	5	1	0.62	60	40			
AMPH									0.15	0.31	0.62

AMK = Amikacin; AMPH = Amphotericin B; Ec = *E coli*; Sa = *S aureus*; Se = *S epidermidis*; Sf = *S faecalis*; Lm = *L monocytogenes*; Pa = *P aeruginosa*; Ca = *C albicans*; Ct = *C tropicalis*; Pv = *P varioti*.

S aureus strains which exhibited resistance to methicillin showed resistance also to cephalosporin-type antibiotics, macrolides and aminoglycosides, and might be reported as 'multi-resisting' strains. The tested compounds were found to be active in the range 2–4 μg/mL against sensitive strains and in the range 2–8 μg/mL against multi-resisting strains (tables VII, VIII).

With respect to antifungal activity, the 1-methyl-4-diazopyrazoles were found to be generally rnore active than the 1-phenyl analogues, especially against *C albicans*. Moreover the 4-nitro derivative **4p** showed activity against all three species tested. No operational scheme was applied to maximize the above activity.

Experimental protocols

General

Melting points were determined on a Büchi-Tottoli apparatus and are uncorrected; IR spectra were recorded with a Jasco IR-810 spectrophotometer as Nujol mulls supported on NaCl disks; ^{1}H-NMR and ^{13}C-NMR spectra were obtained in $CDCl_3$ using a Brüker AC-E 250 MHz spectrometer (using TMS as the internal standard). Microanalyses (C, H, N), performed in the Institut de Chimie Pharmaceutique, Université de Genève, Switzerland, were within ±0.41% of theoretical values.

Table VII. Minimum inhibitory concentration (MIC) in μg/mL against isolates of *S aureus* sensitive to methicillin.

S aureus sensitive strain no	*4l*	*4o*	*4p*
1s	4	2	4
2s	2	2	4
3s	2	2	2
4s	2	2	2
5s	2	2	2

Table VIII. Minimum inhibitory concentration (MIC) in μg/mL against isolates of *S aureus* resistant to methicillin.

S aureus resistant strain no	*4l*	*4o*	*4p*
1r	8	2	4
2r	8	2	4
3r	8	2	4
4r	8	4	4
5r	8	4	4

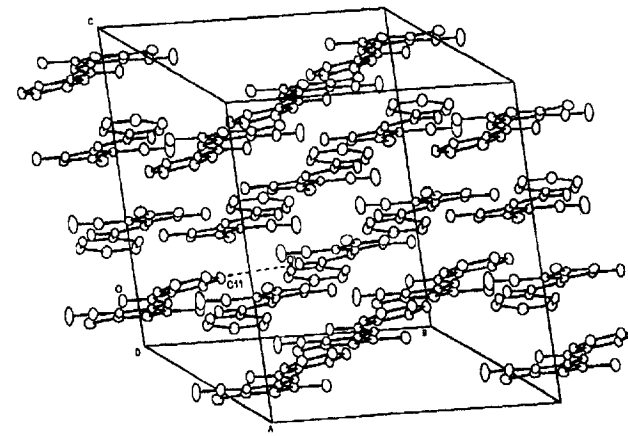


Fig 4. Unit cell content of compound **4l** showing the orientation of the molecules perpendicular to the *c* crystallographic axis. The dashed line indicates the intermolecular C(11)---O' contact. (For the sake of clarity only one contact is indicated and the hydrogen atom positions have been omitted.)

Syntheses

1-R-3-Methyl-5-(R_1-substituted)benzamidopyrazoles, 3i–v

Equimolar amounts (19.8 mmol) of 1-*R*-3-methyl-5-aminopyrazole **1a,b** [10, 11] and the substituted benzoyl chloride **2c–n** (commercially available) in dry chloroform (100 mL) were reacted under reflux for 5 h. After the first hour, triethylamine was added in four portions of 1.39, 0.69, 0.35 and 0.35 mL respectively, at intervals of 1 h. The solution was evaporated under vacuum and the residue was washed with H_2O until a solid was obtained. This was crystallized from the appropriate solvent; yields 50–65% (see table IX).

1-R-3-Methyl-4-diazo-5-(R_1-substituted)benzamido pyrazoles 4i–v

Hydrochloric acid (9 mL) of 37% solution and then potassium nitrite (7.38 g, 86.8 mmol) dissolved in water (3.8 mL) were added at room temperature to a magnetically-stirred solution of **3i–v** (12.4 mmol) in glacial acetic acid (120 mL). The mixture was then stirred for 24 h, after which time, the procedure depended on the compound. For **4i**, the mixture was diluted with water (500 mL) and the resulting solution was extracted with chloroform (3 × 150 mL). The extracts were dried with anhydrous sodium sulphate and evaporated under vacuum. The residue, after standing for several days, gave a product which was washed with a very small amount of cold water, filtered off, and crystallized from cyclohexane; the yield was 27% (see table X). For compounds **4l–v** the reaction mixture was diluted with water (1000 mL) and the solid product that separated was filtered off and crystallized (see table X); yields were 65–80%.

In vitro antimicrobial assays

The testing media used were Iso-Sensitest Agar (Oxoid) for bacteria and Yeast Morphology Agar (Difco) for fungi. A suitable volume of each substance (in a solution of DMSO) was

Table IX. Physical data of 1-*R*-3-methyl-5-(R_1-substituted)benzamido pyrazoles.

Compd	*R*	R_1	*Mp (°C)*	*Crystallization solvent*	*Formula*	*IR(Nujol, cm^{-1})*	
						ν(CO)	*ν(NH)*
3i	CH_3	H	137–38	Benzene	$C_{12}H_{13}N_3O$	1660	3240
3l	CH_3	4-Cl	110–15	Benzene	$C_{12}H_{12}ClN_3O$	1650	3250
3m	CH_3	3,4 Cl_2	119–20	Benzene	$C_{12}H_{11}Cl_2N_3O{\cdot}H_2O$	1655	3100, 3450
3n	CH_3	4-CF_3	134–36	EtOH	$C_{13}H_{12}F_3N_3O$	1700, 1660	3240
3o	CH_3	2,4 Cl_2	155–56	EtOH	$C_{12}H_{11}Cl_2N_3O{\cdot}H_2O$	1670	3480, 3200
3p	CH_3	4-NO_2	171–72	Benzene	$C_{12}H_{12}N_4O_3$	1670	3450, 3260
3q	C_6H_5	H	102–103	Acet/Petr et	$C_{17}H_{15}N_3O$	1665	3445, 3170
3r	C_6H_5	4-Cl	156–57	Benzene	$C_{17}H_{14}ClN_3O$	1660	3460, 3080
3s	C_6H_5	3,4 Cl_2	183–84	EtOH	$C_{17}H_{13}Cl_2N_3O$	1690	3170
3t	C_6H_5	4-CF_3	147–48	EtOH	$C_{18}H_{14}F_3N_3O$	1665	3540, 3100
3u	C_6H_5	2,4 Cl_2	150–51	Benzene	$C_{17}H_{13}Cl_2N_3O$	1655	3230
3v	C_6H_5	4-NO_2	149–50	EtOH	$C_{17}H_{14}N_4O_3$	1690, 1660	3240, 3180

^{1}H NMR (δ) ($CDCl_3$): **3i**: 2.17 (3H, s, CH_3); 3.57 (3H, s, CH_3); 5.97 (1H, s, pyrazole H-4); 7.28–7.87 (4H, a set of signals, C_6H_4); 8.65 (1H, s, exchangeable, NH). **3l**: 2.17 (3H, s, CH_3); 3.56 (3H, s, CH_3); 5.95 (1H, s, pyrazole H-4); 7.37–7.81 (4H, dd, C_6H_4); 9.00 (1H, s, exchangeable, NH). **3m**: 2.13 (3H, s, CH_3) ; 3.52 (3H, s, CH_3); 5.92 (1H, s, pyrazole H-4); 7.44–7.95 (3H, a set of signals, C_6H_3); 8.48 (1H, s, exchangeable, NH). **3n**: 2.23 (3H, s, CH_3); 3.72 (3H, s, CH_3); 6.14 (1H, s, pyrazole H-4); 7.68–8.15 (4H, a set of signals, C_6H_4); 9.30(1H, br s, exchangeable, NH). **3o**: 2.21 (3H, s, CH_3); 3.69 (3H, s, CH_3); 6.13 (1H, s, pyrazole H-4); 7.33–7.70 (3H, a set of signals, C_6H_5); 8.37 (1H, br s, exchangeable, NH). **3p**: 2.18 (3H, s, CH_3); 3.69 (3H, s, CH_3); 6.04 (1H, pyrazole H-4); 8.06–8.27 (4H, dd, C_6H_4); 8.12 (1H, s, NH). **3q**: 2.27 (3H, s, CH_3); 6.45 (1H, s, pyrazole H-4); 7.35–7.70 (9H, a set of signals, C_6H_5 and C_6H_4); 8.42 (1H, br s, exchangeable, 1H). **3r**: 2.27 (3H, s, CH_3); 6.40 (1H, s, pyrazole H-4); 7.30–7.72 (9H, a set of signals, C_6H_5 and C_6H_4); 8.40 (1H, br s, exchangeable, NH). **3s**: 2.24 (3H, s, CH_3); 6.35 (1H s, pyrazole H-4); 7.31–7.78 (8H, a set of signals, C_6H_5 and C_6H_3); 8.53 (1H, s, NH). **3t**: 2.27 (3H, s, CH_3); 6.46 (1H, s, pyrazole H-4); 7.36–7.80 (9H, a set of signals, C_6H_5 and C_6H_4); 8.50 (1H, s, exchangeable, NH). **3u**: 2.33 (3H, s, CH_3); 6.64 (1H, s, pyrazole H-4); 7.31–7.77 (8H, a set of signals, C_6H_5 and C_6H_3). **3v**: 2.34 (3H, s, CH_3); 6.62 (1H, s, pyrazole H-4); 7.40–8.30 (10H, a set of signals, C_6H_5, C_6H_4 and NH).

^{13}C NMR (δ) ($CDCl_3$): **3i**: 13.86 (CH_3-C); 35.22 (CH_3-N); 100.45 (pyrazole C_4); 127.50, 128.77, 132.44 (C_6H_5, $C_{2,3,4,5,6}$); 133.02, 135.73 (C_6H_5, C_1; pyrazole C_3), 147.32 (pyrazole C_5), 166.38 (CO). **3q**: 13.95 (CH_3-C); 99.57(pyrazole C_4); 124.42; 27.08, 127.59, 128.48, 129.70, 132.30 (2 × C_6H_5, 2 × $C_{2,3,4,5,6}$) 133.33, 135.97,138.18 (pyrazole C_3; 2 × C_6H_5, 2 × C_1); 149.58 (pyrazole C_5); 164.31 (CO).

added to 20 mL of molten agar (at 50 °C) and the resulting mixture was poured onto plates and allowed to solidify. The plates were inoculated with a bacterial suspension containing ≈10^6 cfu/mL or a fungal suspension containing ≈10^5 cfu/mL, using a 1 μL calibrated loop. The microbial cultures were incubated at 37 °C for 24 h. The lowest concentration of substance which completely inhibited the growth of the test organism when compared with the growth of a drug-free control (containing the maximum DMSO concentration used) was considered the MIC.

X-ray analysis

Crystals suitable for crystallographic analysis were obtained from ethanol solution. Crystal data are given in table I. Unit cell and intensity data were obtained with a Philips PW1100 diffractometer. Reflections were measured by the θ/2θ scan method, with a scan speed of 1.80°/min, and a scan width of 1.20°; background counts at both ends of the scan, 20 s. Because of the low absorption coefficients no absorption correction was applied to the intensity data. The structures were solved by direct methods [12]. The hydrogen atoms were introduced at fixed positions $d_{C\text{-}H}$ = 0.98 Å and with a unique isotropic thermal parameter of 0.07 Å^2. The non-hydrogen atoms were refined anisotropically. The final Fourier difference maps showed no significant peaks. In all structures the final shift/error ratio in the refinement was less than 0.01. Structure refinement was carried out with SHELXL76 [13] using the scattering factors enclosed therein.

Table X. Physical data of 1-*R*-3-methyl-4-diazo-5-(R_1-substituted)benzamido pyrazoles.

Compd	*R*	R_1	*Mp (°C)*	*Crystallization solvent*	*Formula*	*IR(Nujol, cm^{-1})*	
						ν(CO)	*ν(N_2^+)*
4i	CH_3	H	119–20	Cyclohexane	$C_{12}H_{11}N_5O$	1605, 1620(w)	2150
4l	CH_3	4-Cl	186–87	EtOH	$C_{12}H_{10}ClN_5O$	1600	2150
4m	CH_3	3,4 Cl_2	151–53	EtOH	$C_{12}H_9Cl_2N_5O$	1600	2140
4n	CH_3	4-CF_3	132–33	Benzene	$C_{13}H_{10}F_3N_5O$	1607	2155
4o	CH_3	2,4 Cl_2	203–05	EtOH	$C_{12}H_9Cl_2N_5O$	1600	2155
4p	CH_3	4-NO_2	168–69	Benzene	$C_{12}H_{10}N_6O_3$	1613(w), 1620(w)	2150
4q	C_6H_5	H	123–24	EtOH	$C_{17}H_{13}N_5O$	1610, 1620	2155
4r	C_6H_5	4-Cl	138–39	EtOH	$C_{17}H_{12}ClN_5O$	1605	2140
4s	C_6H_5	3,4 Cl_2	148–49	EtOH	$C_{17}H_{11}Cl_2N_5O$	1616	2130, 2140
4t	C_6H_5	4-CF_3	115	EtOH	$C_{18}H_{12}F_3N_5O$	1610	2160
4u	C_6H_5	2,4 Cl_2	151–52	EtOH	$C_{17}H_{11}Cl_2N_5O$	1610	2150
4v	C_6H_5	4-NO_2	234–35	Benzene	$C_{17}H_{12}N_6O_3$	1610(w)	2145

w = weak band.
^{1}H NMR ($CDCl_3$): **4i** 2.35 (3H, s, CH_3); 3.78 (3H, s, CH_3); 7.41–8.28 (5H, a set of signals, C_6H_5); **4l**: 2.35 (3H, s, CH_3); 3.75 (3H, s, CH_3) 7.35–8.18 (4H, dd, C_6H_4); **4m**: 3.39 (3H, s, CH_3), 3.77 (3H, s, CH_3); 7.46–8.33 (3H, a set of signals, C_6H_3); **4n**: 2.35 (3H, s, CH_3); 3.76 (3H, s, CH_3); 7.64–8.36 (4H, dd, C_6H_4); **4o**: 2.39 (3H, s, CH_3); 3.72 (3H, s, CH_3); 7.24–7.87 (3H, a set of signals, C_6H_3); **4p**: 2.40 (3H, s, CH_3); 3.78 (3H, s, CH_3); 8.16–8.40(14H, dd, C_6H_4); **4q**: 2.23 (3H, s, CH_3); 7.16–8.26 (10H, a set of signals, 2 × C_6H_5); **4r**: 2.36 (3H, s, CH_3); 7.20–8.30 (9H, a set of signals, C_6H_5 and C_6H_4); **4s**: 2.47 (3H, s, CH_3); 7.36–8.26 (8H, a set of signals, C_6H_5 and C_6H_3); **4t**: 2.43 (3H, s, CH_3); 7.30–8.40 (9H, a set of signals, C_6H_5 and C_6H_4); **4u**: 2.47 (3H, s, CH_3); 7.31–7.97 (8H, a set of signals, C_6H_5 and C_6H_3); **4v**: 2.50 (3H, s, CH_3); 7.39–8.37 (9H, a set of signals, C_6H_5 and C_6H_4).
^{13}C NMR ($CDCl_3$): **4i**: 12.09 (CH_3-C); 73.46 (pyrazole C_4); 127.62, 128.74, 131.15 (C_6H_5, $C_{2,3,4,5,6}$); 137.47, 145.07 (C_6H_5, C_1; pyrazole C_3); 155.06 (pyrazole C_5); 175.34 (CO); **4q**: 12.36 (CH_3C); 74.16 (pyrazole C_4); 123.55, 126.73, 128.01, 128.56, 129.06, 131.49 (2 × C_6H_5, 2 × $C_{2,3,4,5,6}$); 137.42, 139.08, 146.01 (2 × C_6H_5, 2 × C_1; pyrazole C_3); 151.66 (pyrazole C_5); 176.16 (CO).

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