

Note

Regioselective reaction : Synthesis and biological activity of some Mannich derivatives

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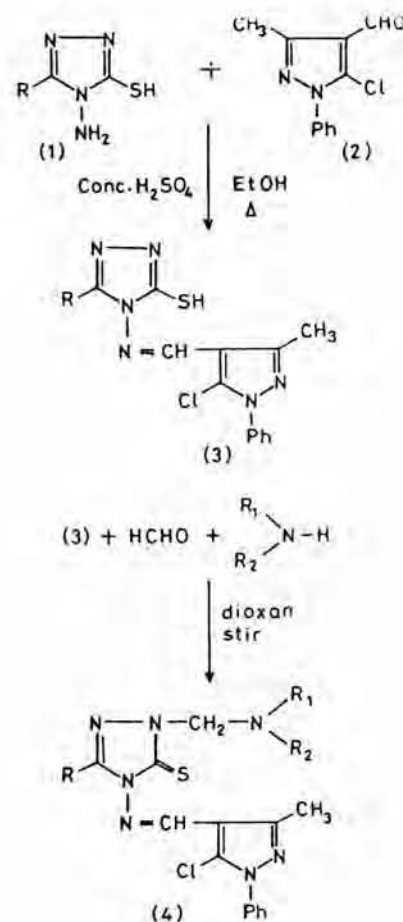
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Received 4 January 1998; accepted (revised) 4 December 1998

The Mannich reaction of 3-(substituted)-4-(1-phenyl-3-methyl-5-chloro-4-pyrazolidine)amino-5-mercapto-1, 2, 4-triazoles **3** with formaldehyde and appropriate amine results in the regioselective formation of 1-aminomethyl-3-(substituted)-4-(1-phenyl-3-methyl-5-chloro-4-pyrazolidine)amino-1, 2, 4-triazole-5-thiones **4**. The structures of new compounds have been established by analytical and spectral data. These compounds have been tested for their antifungal and antibacterial activities.

4-Amino-5-mercapto-1, 2, 4-triazoles are the starting materials for the synthesis of a wide variety of heterocyclic derivatives which are of great importance in medicinal chemistry^{1, 2}. The Mannich bases are associated with potent biological activities such as antibacterial, antifungal, antiviral, antimalarial and CNS depressants^{3, 4}. Similarly the pyrazole nucleus is reported to possess strong bactericidal and fungicidal properties⁵.

Keeping in view of these observations and in continuation of our search for biologically active heterocycles,^{6,7,8} we planned to synthesize the Mannich bases carrying both triazole and pyrazole nuclei, starting from the corresponding 3-(substituted)-4-(1-phenyl-3-methyl-5-chloro-4-pyrazolidene)amino, 5-mercapto 1, 2, 4-triazoles. The Schiff bases **3** on treatment with suitable amines in the presence of formaldehyde underwent Mannich reaction to give the Mannich bases **4** (Scheme I). However, it is interesting to note that the reaction is highly regioselective and furnishes only the *N*-Mannich base and none of the *S*-Mannich derivatives, although the Schiff base **3** can exist in thiol-thione tautomeric equilibrium. 3-Alkyl⁹, aryl¹⁰ or aryloxymethyl¹¹ substituted-4-amino 5-mercapto-1, 2, 4-triazoles and 1-phenyl-3-methyl-5-chloro pyrazole-4-carboxaldehyde¹² were prepared as per the procedures reported in the literature. Condensation of triazole **1** with aldehyde **2**



Scheme I

in ethanol medium using conc. H₂SO₄ as the catalyst gave the Schiff bases **3** in good yields (*cf.* Table I). Formation of Schiff base **3** was confirmed by analytical and spectral data. In a typical example the proton NMR spectrum of the Schiff base (**3a**) exhibited two singlets at δ 2.35 and 2.5 integrating for three protons each due to the methyl groups of triazole and pyrazole moieties. The aromatic protons appeared as a multiplet at δ 7.5-7.8 integrating for five protons. The signal due to N=CH proton appeared at δ 10.16 whereas the thiol proton resonated at δ, 13.75. The NMR spectrum of Schiff base (**3b**) was also in conformity with the assigned structure and the chemical shift are as follows: ¹H NMR(DMSO-*d*₆): δ, 1.3(t, 3H, CH₃), 2.75(q, 2H, CH₂), 2.5(s, 3H, CH₃), 7.35-7.55(m, 5H, Ar-H), 10.4(s, 1H, N=CH), 13.4(s, 1H, SH).

Table I—Characterization data of 3-(substituted)-4-(1-phenyl-3-methyl-5-chloro-4-pyrazolidene)amino-1, 2, 4-triazole-5-thiones **3**.

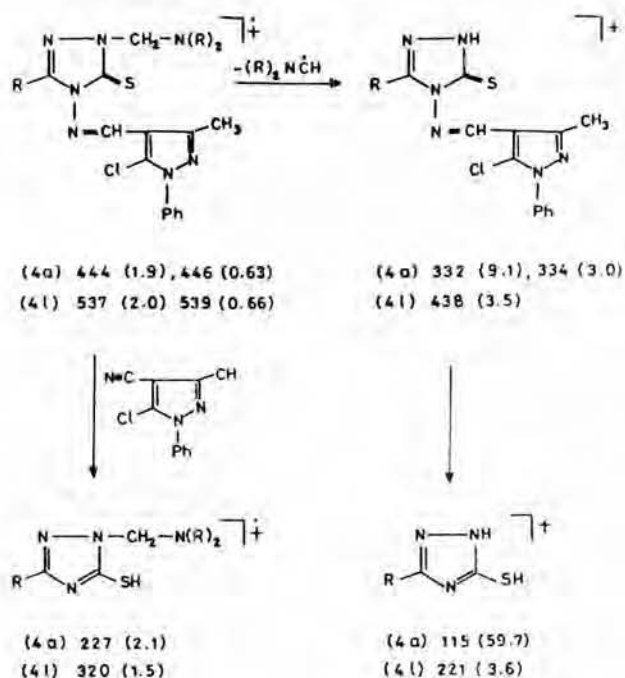
Compd	R	m p °C	Yield (%)	Colour and crystal form	Mol. formula	N
						Found (Calcd) (%)
3a	Methyl	245-46	90	White Micro needles	C ₁₄ H ₁₃ N ₆ SCl	25.27 (25.26)
3b	Ethyl	228-29	89	White powder	C ₁₅ H ₁₅ N ₆ SCl	24.24 (24.24)
3c	Phenyl	247-48	93	Grey coloured micro crystals	C ₁₉ H ₁₅ N ₆ SCl	21.25 (21.29)
3d	<i>p</i> -Cresyloxymethyl	197-99	86	Creamy micro crystals	C ₂₁ H ₁₉ N ₆ OSCl	19.16 (19.15)
3e	<i>p</i> -Chlorophenoxymethyl	195-96	79	Creamy micro crystals	C ₂₀ H ₁₆ N ₆ OSCl ₂	18.32 (18.30)

Formation of *N*-Mannich base rather than *S*-Mannich base was confirmed by studying the proton NMR spectra of Mannich bases. In the proton NMR spectra of these Mannich bases the signal at δ , 5.0-5.2 is typical of the N-CH₂- group. Further, absence of any signal in the region of δ , 4.0-4.5 clearly reveals the absence of S-CH₂ group, thereby indicating that the Mannich reaction did not take place at the sulphur atom. As examples the chemical shifts of **4a** and **4b** are as follows. **4a**: ¹H NMR(CDCl₃): δ 1.7(s, 3H, N-CH₃), 2.4(s, 3H, CH₃) 2.65(s, 3H, pyrazole CH₃), 2.85 [t, 4H, N(CH₂)₂], 3.75 (t, 4H, N(CH₂)₂ of the *N*-methyl side), 5.1(s, 2H, N-CH₂), 7.3-7.6(m, 5H, Ar-H), 10.41(s, 1H, N=CH); **4b**: ¹H NMR(CDCl₃): δ 2.4(s, 3H, triazole-CH₃), 2.6(s, 3H, pyrazole-CH₃), 2.8[t, 4H, N(CH₂)₂], 3.7(t, 4H, O(CH₂)₂), 5.1(s, 2H, N-CH₂), 7.3-7.7(m, 5H, Ar-H), 10.45(s, 1H, N=CH).

The IR spectrum of compounds **4** showed an absorption band at 1120-1150 cm⁻¹ characteristic of the thione group. Another prominent absorption band appeared at 1590-1620 cm⁻¹ for -C=N- moiety. Further evidence for the proposed structure was obtained by recording their mass spectrum. The mass spectrum of Mannich base (**4a**) showed the molecular ion peak at *m/z*, 444, consistent with the molecular formula C₂₀H₂₅ClN₈S, and the chlorine isotope peak was observed at *m/z*, 446. The major fragments were observed at *m/z* 332 (M-R₂NCH), 227(M-A), 115(M-R₂NCH-A).

The mass spectrum of **4l** was also in agreement with the assigned structure.

Biological studies. Among the Mannich bases, six compounds were subjected to both antibacterial and antifungal screening, *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* were the test organisms

**Scheme II**

employed for antibacterial studies, and *C. albicans* and *A. niger* for antifungal studies. Tetracyclin and Griseofulvin were employed as standards. The results of such studies are summarised in **Table III**. Most of the compounds showed significant antifungal activity.

Experimental Section

General. Melting points were determined in open capillary tubes and are uncorrected. Proton NMR spectra were recorded on a 90MHz NMR spectrometer using TMS as internal standard and mass spectra on a Joel-JMS-D300 spectrometer operating at 70eV.

Table II—Characterization data of 1-aminomethyl-3-substituted-4-(1-phenyl-3-methyl-5-chloro-4-pyrazolidene)amino-1, 2, 4-triazole-5-thiones **4**

Compd	R	NR ¹ R ²	mp °C	Yield (%)	Colour and Crystal form	Mol. formula	N
							Found (Calcd) (%)
4a	Methyl	<i>N</i> -Methyl-Piperazine	142 ^a	63	Creamy flakes	C ₂₆ H ₂₅ N ₈ SCl	25.18 (25.19)
4b	Methyl	Morpholine	138 ^a	60	Yellow crystals	C ₁₉ H ₂₂ N ₇ SOC1	22.75 (22.71)
4c	Methyl	Diphenyl amine	168 ^a	50	Light brown crystals	C ₂₇ H ₂₄ N ₇ SCl	19.07 (19.08)
4d	Methyl	<i>p</i> -Chloroaniline	166-70 ^a	45	Yellowish crystals	C ₂₆ H ₁₉ N ₇ SCl ₂	21.32 (21.30)
4e	Ethyl	<i>N</i> -Methyl piperazine	130 ^a	74	White flakes	C ₂₁ H ₂₇ N ₈ SCl	24.41 (24.42)
4f	Ethyl	Morpholine	98-100 ^a	80	Pale yellow flakes	C ₂₆ H ₂₄ N ₇ SOC1	21.96 (21.99)
4g	Ethyl	Diphenyl amine	160 ^a	59	Creamy needles	C ₂₈ H ₂₆ N ₇ SCl	18.59 (18.57)
4h	Phenyl	<i>N</i> -Methyl piperazine	160-62 ^a	72	Brownish crystals	C ₂₅ H ₂₇ N ₈ SCl	22.11 (22.11)
4i	Phenyl	Morpholine micro	168-70 ^b	50	Brownish crystals	C ₂₄ H ₂₄ N ₇ OSCl	19.86 (19.85)
4j	Phenyl	Diphenyl amine	172-74 ^b	50	Brownish crystals	C ₃₂ H ₂₆ N ₇ SCl	17.00 (17.02)
4k	Phenyl	<i>p</i> -Chloroaniline	224-26 ^b	40	Pale yellow micro crystals	C ₂₆ H ₂₁ N ₇ SCl ₂	18.35 ^f (18.35)
4l	<i>p</i> -Cresyloxy-methyl	Morpholine micro crystals	94-98 ^a	45	Brown	C ₂₆ H ₂₈ N ₇ O ₂ SCl	18.26 (18.23)
4m	<i>p</i> -Cresyloxy-methyl	Diphenyl amine	122 ^a	44	Brown crystals	C ₃₄ H ₃₀ N ₇ OSCl	15.80 (15.81)
4n	<i>p</i> -Cresyloxy methyl	<i>p</i> -Chloroaniline	112-15 ^a	52	White crystals	C ₂₈ H ₂₅ N ₇ OSCl ₂	16.93 (16.95)
4o	<i>p</i> -Chloro phenoxy methyl	<i>N</i> -Methyl Piperazine	98-100 ^a	50	White crystals	C ₂₆ H ₂₈ N ₈ OSCl ₂	19.64 (19.61)
4p	<i>p</i> -Chloro phenoxy methyl	<i>p</i> -Chloroaniline	116-18 ^a	66	Yellowish crystals	C ₂₇ H ₂₂ N ₇ OSCl ₃	16.39 (16.37)

* Solvent of recrystallisation (a) EtOH, (b) dioxane.

Synthesis of Schiff bases 3: General procedure. An equimolecular mixture of triazole **1**, (0.01 mole) and 1-phenyl-3-methyl-4-chloro-pyrazol-3-carboxaldehyde in ethanol (15mL) and con.H₂SO₄ (0.4mL) was refluxed on a water-bath for 1-2 hr. The solid separated was collected by filtration, dried and recrystallised from dioxane. The yields, melting points and other characterisation data of these compounds are given in **Table I**.

General procedure for the preparation of 1-aminomethyl-3-substituted-4-(1-phenyl-3-methyl-5-chloro-4-pyrazolidene) amino-1, 2, 4-triazole-5-thiones 4. A solution of appropriate Schiff base **3** (0.01 mole), formaldehyde (0.01 mole) and the amine (0.01mole) in dioxane was stirred for 24 hr and left overnight in a freezer. The solution was poured on to crushed ice. The resulting solid was collected by filtration, dried and recrystallised from suitable

Table III—Antibacterial and antifungal activity data of compounds **4**

Compd	Antibacterial (inhibition zone in mm)				Antifungal (inhibition zone in mm)	
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>P.aeruginosa</i>	<i>E.coli</i>	<i>C.albicans</i>	<i>A.niger</i>
4a	—	8.35	8.55	8.30	—	—
4b	—	8.90	8.55	8.50	—	10.00
4d	10.5	9.65	9.15	9.50	8.40	—
4j	—	9.25	9.25	9.00	9.85	10.05
4n	9.15	10.60	—	9.75	9.45	11.60
4p	8.25	11.05	11.20	10.25	—	12.15
DMFControl	—	—	—	—	—	—
Standard (Tetracyclin)	21.80	25.20	21.20	20.25	—	—
Standard (Griseofulvin)	—	—	—	—	14.00	14.00

Index for biological activity: Disc size = 5.5mm, Duration 24hr, Sample concentration = 100 µg/disc; *S.aureus* = *Staphylococcus aureus*; *P.aeruginosa* = *Pseudomonas aeruginosa*; *C.albicans* = *Candida albicans*; *B.subtilis* = *Bacillus subtilis*; *E.coli* = *Escherichia coli*; *A.niger* = *Aspergillus niger*

solvent. The characterization data of **4** are given in **Table II**.

Acknowledgement

The authors are thankful to Head, RSIC, CDRI, Lucknow for the analytical and spectral data. One of them (P G) is grateful to Mangalore University for financial assistance.

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