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Short communication

Regioselective reaction: Synthesis, characterization and pharmacological studies of some new Mannich bases derived from 1,2,4-triazoles

Arun M. Isloor [a,*], Balakrishna Kalluraya [b], Prashanth Shetty [c]

[a] Department of Chemistry, National Institute of Technology-Karnataka, Surathkal, Mangalore 575 025, India
[b] Department of P.G Studies and Research in Chemistry, Mangalore University, Mangalagangothri 574 199, India
[c] Department of Pharmacology, NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Mangalore 574160, India

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ABSTRACT

In the present investigation, a series of new 4[(3-substituted-1*H*-pyrazol-4-yl)methyleneamino]-5-substituted-2-[(4-methylpiperzine-1-yl)methyl]-2*H*-1,2,4-triazole-3(4*H*)-thiones (**4**) were synthesized by the aminomethylation of 4-(3-substituted-1*H*-pyrazol-3-yl)methyleneamino-5-substituted-4*H*-1,2,4-triazole-3-thiols (**3**) with formaldehyde and *N*-methylpiperzine. These newly synthesized Schiff and Mannich bases were characterized by IR, ^{1}H NMR, mass spectral data and elemental analyses. These compounds were screened for their antibacterial and antifungal activity. Some of the compounds were found to exhibit significant antimicrobial activity.

1. Introduction

The 1,2,4-triazole and its derivatives were reported to exhibit various pharmacological activities such as antimicrobial, analgesic, anti-inflammatory, anticancer and antioxidant properties [1–4]. Some of the present day drugs such as Ribavirin (antiviral agent), Rizatriptan (antimigraine agent), Alprazolam (anxiolytic agent), Fluconazole and Itraconazole (antifungal agents) are the best examples for potent molecules possessing triazole nucleus. Further 1,2-pyrazole derivatives were also found to possess various biological activities [5–7]. Many literatures have shown that Mannich bases possess potent biological activities such as antibacterial, antifungal, anti-inflammatory, antimalarial and pesticide properties [8–11]. Few Mannich bases derived from 1,2,4-triazoles carrying *N*-methylpiperzine substituent were biologically active [12,13]. In view of these facts and as continuation of our research on pharmaceutically important heterocycles [14–16], hereby we report the synthesis of a new series of Mannich bases containing both 1,2,4-triazoles and pyrazole skeletons.

2. Chemistry

3-Substituted-4-amino-5-mercapto-1,2,4-triazoles (**1**) were synthesized as reported in the literature [17,18] and 3-substituted-1*H*-pyrazole-4-carbaldehyde (**2**) were synthesized by the Wilsmayer Haack reaction of semicarbazones [19]. Condensation of amino mercapto triazoles (**1**) with various 3-substituted-1*H*-pyrazole-4-carbaldehyde (**2**) in the presence of concentrated sulphuric acid in ethanol–dioxane mixture yielded Schiff bases (**3**). Mannich bases (**4**) were synthesized by reacting Schiff bases (**3**) with *N*-methylpiperzine in the presence of formaldehyde and ethanol–dioxane medium. Newly synthesized compounds (**3** and **4**) were characterized by IR, NMR, mass spectral and C, H, N analyses (Scheme 1; Tables 1 and 2).

3. Results and discussion

The antibacterial and antifungal screening revealed that some of the tested compounds showed good inhibition at 3 μg/ml concentration. The antibacterial screening indicated that among the tested compounds, **4h** showed excellent activity against all the tested bacterial strains, namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The remaining compounds found to be active at higher concentrations, e.g., 6 and 12.5 μg/ml.

* Corresponding author. Fax: +91 824 2474033.
E-mail address: isloor@yahoo.com (A.M. Isloor).

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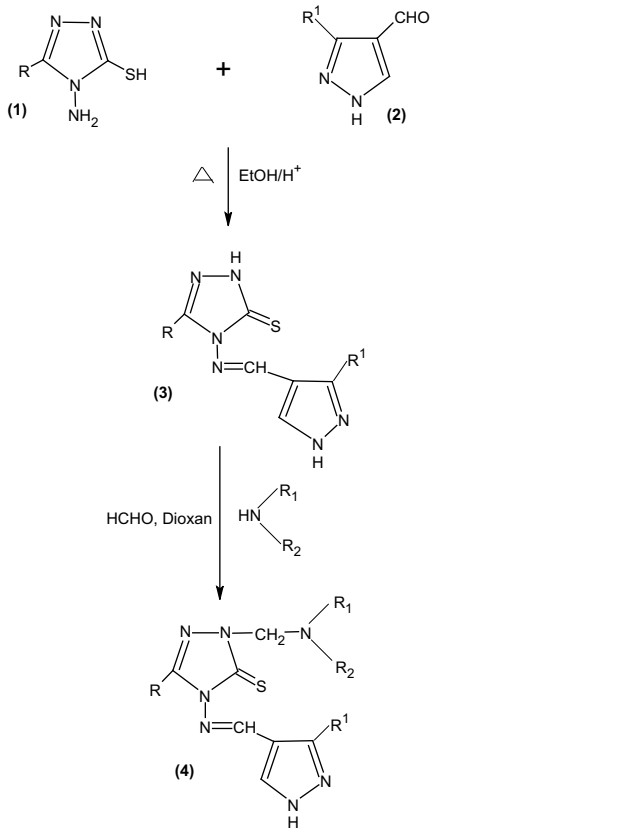

Scheme 1.

The antifungal screening revealed that among the tested compounds, **4h** and **4l** showed excellent activity against fungal strain *Candida albicans* at 3 μg/ml concentration. However, remaining compounds were active at 6 μg/ml concentration.

4. Conclusions

A series of novel Mannich bases, namely 4[(3-substituted-1*H*-pyrazol-4-yl)methyleneamino]-5-substituted-2-[(4-methylpiperzine-1-yl)methyl]-2*H*-1,2,4-triazole-3(4*H*)-thiones were synthesized and characterized by NMR, mass spectrometry and IR studies. All the newly synthesized compounds were screened for their antibacterial and antifungal activities by the method of minimum inhibitory concentration (MIC). Antimicrobial study reveals that compound **4h** having *p*-chloro substitution exhibited maximum inhibition against all the tested microorganisms. Similarly, compounds **4k** and **4l** also showed significant activity against microorganism *P. aeruginosa*, which have *p*-methoxy and *p*-methyl substituent on the phenyl ring, respectively. All remaining compounds showed moderate inhibition. 1,2,4-Triazole nucleus is one of the active components present in all molecules. Also the presence of *N*-methylpiperazine moiety is contributing to the net biological activity of the system (Table 3).

5. Experimental

5.1. Chemistry

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. ^{1}H NMR spectra were recorded on a Perkin–Elmer EM 300 MHz spectrometer using TMS as internal standard. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Purity of the compounds was checked by TLC silica coated plates obtained from Merck.

5.2. 4-[(3-Substituted-1H-pyrazol-3-yl)methyleneamino]-5-substituted-4H-1,2,4-triazole-3-thiols (3)

To a suspension of various 3-substituted-1*H*-pyrazole-4-carbaldehyde (**2**; 10 mmol) in ethanol–dioxane mixture (2:1), an equimolar amount of the corresponding 3-substituted-4-amino-5-mercapto-1,2,4-triazole (**4**) was added. The suspension was heated till a clear solution was obtained. Then 2–3 drops of concentrated sulphuric acid was added and solution was refluxed on water bath

Table 1
Characterization data of 4-[(3-substituted-1*H*-pyrazol-3-yl)methyleneamino]-5-substituted-4*H*-1,2,4-triazole-3-thiols.

Compound	R	R^1	Melting point (°C)	Yield (%)	Molecular formula (mol. wt.)	Analyses (%) found (calculated)		
						C	H	N
3a	CH_3	CH_3	207–208	74	$C_8H_{10}N_6S$ (222)	43.21 (43.24)	4.53 (4.50)	37.82 (37.83)
3b	CH_3	C_6H_5	218–220	65	$C_{13}H_{12}N_6S$ (284)	54.90 (54.92)	4.25 (4.22)	29.56 (29.57)
3c	CH_3	4-OCH_3 C_6H_4	259–260	55	$C_{14}H_{14}N_6OS$ (314)	53.52 (53.50)	4.46 (4.45)	26.78 (26.75)
3d	CH_3	4-CH_3 C_6H_4	236–238	58	$C_{14}H_{14}N_6S$ (298)	56.36 (56.37)	4.65 (4.69)	28.15 (28.18)
3e	C_3H_7	C_6H_5	191–192	55	$C_{15}H_{16}N_6S$ (312)	57.71 (57.69)	5.13 (5.12)	26.90 (26.92)
3f	C_3H_7	4-OCH_3 C_6H_4	154–155	62	$C_{16}H_{18}N_6OS$ (342)	56.18 (56.14)	5.25 (5.26)	24.54 (24.56)
3g	C_3H_7	4-CH_3 C_6H_4	219–220	60	$C_{16}H_{18}N_6S$ (326)	58.90 (58.89)	5.53 (5.52)	25.77 (25.76)
3h	C_3H_7	4-Cl C_6H_4	213–214	70	$C_{15}H_{15}ClN_6S$ (346)	51.95 (51.95)	4.30 (4.32)	24.23 (24.24)
3i	C_6H_5	CH_3	228–229	53	$C_{13}H_{12}N_6S$ (284)	54.93 (54.92)	4.23 (4.22)	29.59 (29.57)
3j	C_6H_5	C_6H_5	212–213	83	$C_{18}H_{14}N_6S$ (346)	62.40 (62.42)	4.03 (4.04)	24.30 (24.27)
3k	C_6H_5	4-OCH_3 C_6H_4	229–230	56	$C_{19}H_{16}N_6OS$ (376)	60.63 (60.63)	4.27 (4.25)	2.34 (2.34)
3l	C_6H_5	4-CH_3 C_6H_4	208–209	80	$C_{19}H_{16}N_6S$ (360)	63.32 (63.33)	4.46 (4.44)	23.35 (23.33)

IR (KBr, cm^{-1}): **3a**, 3120 and 3050 (NH/SH str), 1615 (C=N), 2835 (C–H str), 1282 (C=S), 1030 (C–S str); **3j**, 3115 and 3018 (NH/SH str), 2932 (C–H str), 1232 (C=S), 1035 (C–S str). ^{1}H NMR ($CDCl_3$, 300 MHz): **3a**, δ 11.58 (s, 1H, NH of pyrazole), 7.9 (s, 1H of pyrazole, 5H), 2.45 (s, 3H methyl of pyrazole), 8.07 (s, 1H, N=C–H), 1.95 (s, 3H triazole); **3b**, δ 13.63 (br, 1H, SH), 2.24 (S, 3H, CH_3), 7.3–7.9 (m, 6H, Ar–H and pyrazole 5H), 9.50 (s, 1H, N=CH), 12.9 (s, 1H, NH); **3c**, δ 11.58 (s, 1H, NH of pyrazole), 7.904 (s, 1H, 5H of pyrazole), 3.7 (s, 3H of O–CH_3), 8.07 (s, 1H, N=C–H), 1.95 (s, 3H of triazole); **3g**, δ 11.58 (s, 1H, NH of pyrazole), 7.904 (s, 1H of pyrazole, 5H), 7.014–8.014 (m, 7H of *p*-methyl phenyl), 8.07 (s, 1H, N=C–H); **3h**, δ 11.58 (s, 1H, NH of pyrazole), 8.07 (s, 1H of pyrazole, 5H), 7.014–8.014 (m, 4H of *p*-chlorophenyl), 8.07 (s, 1H, N=C–H); **3k**, δ 3.84 (s, 3H, OCH_3), 7.0 (d, 2H, *ortho* protons of *p*-anisyl, $J = 9$ Hz), 7.66 (d, 2H, *meta* protons of *p*-anisyl, $J = 9$ Hz), 7.4–8.0 (m, 6H, Ar–H and pyrazole 5H), 9.58 (s, 1H, N=CH), 12.9 (br s, 1H, NH), 13.97 (br s, 1H, SH); **3i**, δ 3.10 (s, 3H, CH_3), 13.85 (br s, 1H, SH), 7.35–8.0 (m, 6H, Ar–H and pyrazole 5H), 9.65 (s, 1H, N=CH), 12.8 (br s, 1H, NH). Mass: **3j**, *m/z* 346 (M^+), 176 (3-mercapto-4(*H*)-1,2,4-triazole), 169 (phenylpyrazolenitrile), 77 (phenyl nucleus); **3a**, *m/z* 222 (M^+), 115 (3-mercapto-4-(*H*)-1,2,4-triazole), 106 (methylpyrazolenitrile), 80 (loss of CN^- from 3-mercapto-4(*H*)-1,2,4-triazole).

Table 2
Characterization data of 4[(3-substituted-1*H*-pyrazol-4-yl)methyleneamino]-5-substituted-2-[(4-methylpiperzine-1-yl)methyl]-2*H*-1,2,4-triazole-3(4*H*)-thiones.

Compound	R	R^1	Melting point (°C)	Yield (%)	Molecular formula (mol. wt.)	Analyses (%) found (calculated)		
						C	H	N
4a	CH_3	CH_3	155–157	74	$C_{14}H_{22}N_8S$ (334)	50.34 (50.29)	6.34 (6.58)	33.45 (33.53)
4b	CH_3	C_6H_5	175–176	65	$C_{19}H_{24}N_8S$ (396)	57.54 (57.57)	6.08 (6.06)	28.30 (28.28)
4c	CH_3	4-OCH_3 C_6H_4	202–204	55	$C_{20}H_{26}N_8OS$ (426)	56.39 (56.33)	6.14 (6.10)	26.32 (26.29)
4d	CH_3	4-CH_3 C_6H_4	185–187	58	$C_{20}H_{26}N_8S$ (410)	58.56 (58.53)	6.30 (6.34)	27.30 (27.31)
4e	C_3H_7	C_6H_5	134–136	55	$C_{21}H_{28}N_8S$ (424)	59.40 (59.43)	6.35 (6.60)	26.42 (26.41)
4f	C_3H_7	4-OCH_3 C_6H_4	111–112	62	$C_{22}H_{30}N_8OS$ (454)	58.18 (58.14)	6.58 (6.60)	24.56 (24.66)
4g	C_3H_7	4-CH_3 C_6H_4	182–184	60	$C_{22}H_{30}N_8S$ (438)	60.30 (60.27)	6.90 (6.84)	25.56 (25.57)
4h	C_3H_7	4-Cl C_6H_4	174–175	70	$C_{21}H_{27}ClN_8S$ (458)	55.02 (55.02)	5.75 (5.89)	24.45 (24.45)
4i	C_6H_5	CH_3	179–181	53	$C_{19}H_{24}N_8S$ (396)	53.08 (53.03)	7.20 (7.18)	30.98 (30.93)
4j	C_6H_5	C_6H_5	167–169	83	$C_{24}H_{26}N_8S$ (458)	62.93 (62.88)	5.50 (5.67)	24.48 (24.45)
4k	C_6H_5	4-OCH_3 C_6H_4	159–161	56	$C_{25}H_{28}N_8OS$ (488)	61.38 (61.47)	5.68 (5.73)	22.90 (22.95)
4l	C_6H_5	4-CH_3 C_6H_4	136–138	80	$C_{25}H_{28}N_8S$ (472)	63.48 (63.55)	5.97 (5.93)	23.67 (23.72)

IR (KBr, cm^{-1}): **4a**, 3400 (NH str), 1612 (C=N), 2800 (CH str), 1280 (C=S), 1033 (C–S str), 800 (Ar–H str); **4e**, 3390 (NH str), 1610 (C=N), 2792 (CH str), 1280 (C=S), 1030 (C–S str), 810 (Ar–H str). ^{1}H NMR ($CDCl_3$, 300 MHz): **4a**, δ 5.17 (s, 2H, N–CH_2), 1.58 (s, 3H, N–CH_3), 2.2 (s, 3H, CH_3), 2.8 (t, 4H, CH_2–N–CH_2), 3.6 (t, 4H, CH_2–N–CH_2), 7.12–8.2 (m, 6H, Ar–H and pyrazole 5H), 10.18 (s, 1H, N=CH), 12.63 (br s, 1H, NH); **4b**, δ 11.58 (s, 1H, NH of pyrazole), 7.904 (s, 1H of pyrazole, 5H), 7.35–7.96 (m, 5H of phenyl), 7.48 (s, 1H, N=C–H), 1.94 (s, 3H of methyl), 5.40 (s, 2H N–CH_2–N), 2.23 (s, 3H CH_3 of piperzine), 2.38–2.87 (m, 8H of piperzine); **4g**, δ 11.58 (s, 1H, NH of pyrazole), 7.904 (s, 1H, pyrazole 5H), 7.35–7.96 (m, 7H of *p*-methyl phenyl), 2.3 (s, 3H, CH_3 of *p*-phenyl), 7.48 (s, 1H, N=C–H), 5.40 (s, 2H, N–CH_2–N), 2.23 (s, 3H, CH_3 of piperzine), 2.38–2.87 (m 8H piperzine); **4j**, δ 1.8 (s, 3H, N–CH_3), 2.7 (t, 4H, CH_2–N–CH_2), 3.5 (t, 4H, CH_2–N–CH_2), 5.08 (s, 2H, N–CH_2–N), 7.0–8.8 (m, 10H, Ar–H and pyrazole 5H), 9.95 (s, 1H, N=CH), 12.85 (br s, 1H, pyrazole NH); **4k**, δ 11.58 (s, 1H, NH of pyrazole), 7.904 (s, 1H, pyrazole 5H), 7.35–7.96 (m, 7H, *p*-methoxy phenyl), 3.7 (s 3H, O–CH_3 of *p*-methoxy phenyl), 7.48 (s, 1H, N=C–H), 8.2–7.3 (m, 5H of phenyl), 5.40 (s, 2H N–CH_2–N), 2.23 (s, 3H, CH_3 of piperzine), 2.38–2.87 (m, 8H of piperzine). Mass: **4c** *m*/*z* 426 (M^+), 113 (*N*-methylpiperzinomethyl cation), 77 (phenyl nucleus), 103 (3-mercapto-4(*H*)-1,2,4 triazole), 200 (*p*-methoxyphenylpyrazolenitrile); **4g** *m*/*z* 438 (M^+), 113 (*N*-methylpiperzinomethyl cation), 77 (phenyl nucleus), 143 (3-mercapto-4-(*H*)-1,2,4-triazole), 183 (*p*-methylphenylpyrazolenitrile).

for 7–8 h. Completion of reaction was monitored by TLC. After cooling it for overnight, the precipitated solid was filtered off and recrystallized from a mixture of hot ethanol–dioxane (2:1) to afford the title compounds.

5.3. 4[(3-Substituted-1H-pyrazol-4-yl)methyleneamino]-5-substituted-2-[(4-methylpiperzine-1-yl)methyl]-2H-1,2,4-triazole-3(4H)-thiones (**4**)

The Schiff base (**3**; 10 mmol) was dissolved in a mixture of absolute ethanol–dioxane (2:1) mixture. Then formaldehyde (40%, 1.5 ml) and *N*-methylpiperzine (10 mmol) in absolute ethanol were introduced to this solution. The mixture was stirred for 5–6 h and kept overnight at room temperature. The solid separated was collected by filtration and recrystallized from a mixture of ethanol–dioxane (2:1) to afford title compounds (**4**).

Table 3
Antibacterial and antifungal activity data of 4[(3-substituted-1*H*-pyrazol-4-yl)methyleneamino]-5-substituted-2-[(4-methylpiperzine-1-yl)methyl]-2*H*-1,2,4 triazole -3(4*H*)-thiones.

Compound no.	Antibacterial activity data in MIC (μg/ml)				Antifungal activity data in MIC (μg/ml)
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*
4a	12.5	6	6	6	6
4b	6	6	12.5	6	6
4c	6	6	6	1.5	6
4d	12.5	6	6	12.5	6
4e	6	6	12.5	12.5	6
4f	6	12.5	12.5	6	6
4g	6	12.5	6	6	6
4h	3	3	3	3	3
4i	12.5	12.5	6	12.5	6
4j	6	12.5	6	6	6
4k	6	6	6	3	6
4l	6	12.5	6	3	3
Furacin (std)	12.5	12.5	6	12.5	Flucanazol (std) 6
DMF (control)	–	–	–	–	–

5.4. Pharmacology

5.4.1. Antimicrobial studies

All the newly synthesized Mannich bases were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. For antifungal, *C. albicans* was used as organism. Both microbial studies were assessed by minimum inhibitory concentration (MIC) by serial dilution method [20]. For this the compound whose MIC has to be determined is dissolved in serially diluted DMF. Then a standard drop of the culture prepared for the assay is added to each of the dilutions, and incubated for 16–18 h at 37 °C. MIC is the highest dilution of the compound, which shows clear fluid with no development of turbidity.

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