Heterocyclic Letters
Vol. 9| No.3|279-288|May-July|2019
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

REGIOSELECTIVE REACTION: SYNTHESIS OF 1, 2, 4-TRIAZOLE BASED MANNICH BASES AND THEIR BIOLOGICAL ACTIVITY

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Abstract: In the present paper we describe the regioselective synthesis of novel series of 4[(3-substituted-1H-pyrazol-4-yl)methyleneamino]-5-substituted-2-[(4-methylpiperzine-1-yl)methyl]-2H-1,2,4-triazole-3(4H)-thiones (**4a-l**) by the amino-methylation of 4-(3-substituted-1H-pyrazol-3-yl)methyleneamino-5-substituted-4H-1,2,4-triazole-3-thiols (**3a-f**) with formaldehyde and substituted piperazine. The structures of the newly synthesized Schiff and Mannich bases were confirmed on the basis of spectral and analytical data. Further synthesized compounds were screened for their antibacterial, antifungal and anthelmintic activity. Some of the synthesized compounds were found to exhibit significant activity comparable with that of the standard.

Keywords: Regioselectivity, Mannich Bases, Triazole, Antimicrobial, Anthelmintic activity.

1.0: Introduction.

Nitrogen-containing heterocycles are attracting the synthetic chemists because of the wide variety of biological properties associated with them. There are number of heterocycles possessing nitrogen moiety among those triazole and pyrazole stand unique due to its varied pharmacological activity. 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 [I-IV]. Some of the triazole bearing drugs such as Ribavirin (antiviral agent), Rizatriptan (antimigraine agent), Alprazolam (anxiolytic agent), Fluconazole and Itraconazole (antifungal agents) are examples of potent molecules possessing a triazole ring [V-VII]. Further pyrazole derivatives were also found to possess various biological activities [VIII–IX]. Many studies have shown that Mannich bases have possessed potent biological characteristics such as antibacterial, antifungal, anti-inflammatory, antimalarial and pesticide properties [X-XIII]. Few Mannich bases derived

from 1,2,4-triazoles carrying substituted piperazine derivative were biologically active [XIV, XV]. In addition, there are some studies on electronic structures and the tautomeric equilibrium of heterocyclic thione derivatives [XVI-XIX].

In view of these findings, we report the synthesis, characterization and antibacterial, antifungal and anthelmintic activities some 4-(1-aryl-3-phenyl-1*H*-pyrazol-4-yl]methylidene]amino)-5-methyl-4H-1,2,4-triazole-3-thiols and their Mannich bases.

2.0: Experimental.

2.1: Material, methods, and instrumentation.

All the chemicals were purchased from Sigma-Aldrich or Hi-Media and used after distillation/ recrystallization. The melting points of the newly synthesized compounds were determined in open capillaries and are uncorrected. The IR spectra were recorded on a SHIMADZU FT-IR spectrophotometer in KBr pellet. The ^{1}H-NMR spectra were recorded on a Bruker AC 300F (300/500MHz) NMR spectrometer using DMSO-d_6 as solvent and TMS as an internal standard. All chemical shift values are expressed in δ, scale downfield from TMS and proton signals are indicated as s = singlet, d = doublet, t = triplet, m = multiplet. Mass spectra were recorded on a Jeol JMS-D300 mass spectrometer operating at 70eV. The purity of all compounds was checked and confirmed by TLC.

2.2: General procedure for the preparation of 3-methyl-4-[(1-aryl-3-phenyl-4-pyrazolidene)amino-5-mercapto-1,2,4-triazoles (Schiff bases) (3a-f):

A solution of 3-substituted-4-amino-5-mercapto-1,2,4-triazole **(1)** (0.01mol) in absolute ethanol (25mL) was added to 1-aryl-3-phenyl-1H-pyrazole-4-carbaldehyde **(2a-b)** (0.01mol). Concentrated sulfuric acid (0.8mL) was added to this reaction mixture and contents were refluxed for 4 hours. The solid product separated was collected by filtration, dried and recrystallized from suitable solvent. The characterization data of the synthesized compound (**3a-f**) are given in (**Table-1**).

Table 1: Characterization data of 3-methyl-4-[(1-aryl-3-phenyl-4-pyrazolidene)amino-5-mercapto-1,2,4-tiazoles (3a-f) (Schiff bases).

Comp. No.	R_1	R_2	Yield (%) MP (^{o}C)	Molecular Formula	Colour and crystal nature	Analysis (%) found (Calculated)		
						C	H	N
3a	H	H	78 176-78	$C_{18}H_{14}N_6S$	White crystals	64.29 (62.41)	2.99 (4.07)	25.65 (24.26)
3b	H	CH_3	68 201-03	$C_{19}H_{16}N_6S$	White flakes	62.93 (63.31)	3.01 (4.47)	24.03 (23.32)
3c	H	C_3H_7	75 162-64	$C_{21}H_{20}N_6S$	White crystals	62.01 (64.92)	4.55 (5.19)	23.01 (21.63)
3d	2,4-dinitro	H	68 205-07	$C_{18}H_{12}N_8O_4S$	Red shining crystals	47.56 (49.54)	2.08 (2.77)	22.98 (25.68)
3e	2,4-dinitro	CH_3	72 222-25	$C_{19}H_{15}N_8O_4S$	Red shining crystals	49.56 (50.66)	2.95 (3.13)	22.66 (24.88)
3f	2,4-dinitro	C_3H_7	66 234-36	$C_{21}H_{18}N_8O_4S$	Red shining crystals	50.21 (52.71)	3.01 (3.79)	21.43 (23.42)

Solvent for recrystallization: EtOH + Dioxane

2.2.1: 4-{[1,3-Diphenyl-1*H*-pyrazol-4-yl)methylidene]amino}-4*H*-1,2,4-triazole-3-thiol: (**3a**).
IR (KBr) ν cm^{-1}: 3300 (NH-str.); 3106 (Ar-H-str.); 1590(-N=CH- str.); ^{1}H NMR (500MHz), (solvent $CDCl_3$, δ in ppm): 7.71-7.52 (m, 10H, Ar-H); 8.81 (s, 1H, triazole-5H) 9.000 (s, 1H, pyrazole-5H); 9.200 (s, 1H, –N=CH-); 13.103 (s, 1H, triazole-SH). MS m/z; 346 (M^+ + 1).
2.2.2: 4-{(1,3-Diphenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-methyl-4*H*-1,2,4-triazole-3-thiol (**3b**).
IR (KBr) ν cm^{-1}: 3302 (NH-str.); 3044 (Ar-H-str.); 1592(-N=CH- str.); ^{1}H NMR (500MHz); (solvent $CDCl_3$, δ in ppm): 2.82 (s, 3H, CH_3); 7.72-7.52 (m, 10H, Ar-H); 9.20 (s, 1H, pyrazole-5H); 9.40 (s, 1H, –N=CH-); 13.10 (s, 1H, triazole-SH). MS m/z; 360 (M^+ + 1).
2.2.3: 4-{[1,3-Diphenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-propyl-4*H*-1,2,4-triazole-3-thiol (**3c**).
IR (KBr) ν cm^{-1}: 3301 (NH-str.); 3043(Ar-H-str.); 1591(-N=CH- str.); ^{1}H NMR (500MHz), (solvent $CDCl_3$, δ in ppm): δ, 2.11 (t, 3H, CH_3); 2.21 (m, 2H, CH_2); 2.32 (t, 2H, CH_2); 7.71-7.51 (m, 10H, Ar-H); 9.00 (s, 1H, pyrazole-5H); 9.21 (s, 1H, –N=CH-); 13.10 (s, 1H, triazole-SH). MS m/z; 388 (M^+ + 1).
2.2.4: 4-{[1-(2,4-Dinitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl]methylidene}amino)-4*H*-1,2,4-triazole-3-thiol (**3d**).
IR (KBr) ν cm^{-1}: 3300 (NH-str.); 2955 (CH- str.); 1585(-N=CH- str.); ^{1}H NMR (500MHz); (solvent $CDCl_3$, δ in ppm): 7.70-7.50 (m, 8H, Ar-H); 8.81 (s, 1H, triazole-5H), 9.10 (s, 1H, pyrazole-5H); 9.20 (s, 1H, –N=CH-); 13.10 (s, 1H, triazole-SH). MS m/z; 436 (M^+ + 1).
2.2.5: 4-{[1-(2,4-Dinitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl]methylidene}amino)-5-methyl-4*H*-1,2,4-triazole-3-thiol(**3e**).
IR (KBr) ν cm^{-1}: 3303(NH-str.); 2988 (CH-str.); 1593(-N=CH- str.); ^{1}H NMR (500MHz); (solvent $CDCl_3$, δ in ppm): 2.81 (s, 3H, CH_3); 7.73-7.52 (m, 8H, Ar-H); 9.00 (s, 1H, pyrazole-5H); 9.20 (s, 1H,–N=CH-); 13.10 (s, 1H, triazole-SH). MS m/z; 450 (M^+ + 1).
2.2.6: 4-{[1-(2,4-Dinitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl]methylidene}amino)-5-propyl-4*H*-1,2,4-triazole-3-thiol (**3f**).
IR (KBr) ν cm^{-1}: 3304 (NH- str.); 2970 (CH- str.); 1590(-N=CH- str.); ^{1}H NMR (500MHz); (solvent $CDCl_3$, δ in ppm): 2.10 (t, 3H, CH_3); 2.21 (m, 2H, CH_2); 2.32 (t, 2H, CH_2); 7.71-7.52 (m, 8H, Ar-H); 9.00 (s, 1H, pyrazole-5H); 9.20 (s, 1H, –N=CH-); 13.10 (s, 1H, triazole-SH). MS m/z; 478 (M^+ + 1);

2.3:General procedure for the preparation of Mannich bases (4a-h).

A mixture of Schiff bases **(3a-f)** (0.01mol) and formaldehyde 40% (1.5mL) in ethanol-dioxane mixture was taken in 50 mL round bottomed flask and to this solution, suitable amine (0.01mol) in ethanol (20mL) was added and stirred at room temperature. The solid product gets separated within half an hour. The stirring was continued for another four hours. Finally, the product was collected by filtration, washed with ethanol and dried. It was further purified by recrystallization from a suitable solvent. The characterization data of these newly synthesized Mannich bases (**4a-l**) are given in (**Table 2**).

Table-2: Characterization data of 1-substituted-aminomethyl-3-methyl-4-(1-aryl-3-phenyl-4-pyrazolidene)amino-1,2,4-tiazol-5-thiones (Mannich bases) (4a-l).

Comp. No.	R_1	R	X	Yield (%) MP (°C)	Molecular Formula	Colour and crystal nature	Analysis (%) found (Calculated)		
							C	H	N
4a	H	H	CH_2	69 145-47	$C_{24}H_{25}N_7S$	Light brown Crystals	62.68 (64.99)	6.21 (5.88)	20.15 (22.10)
4b	H	H	NCH_2CH_3	75	$C_{25}H_{28}N_8S$	Light	61.25	5.01	21.88

				163-65		brown flakes	(63.53)	(5.97)	(23.71)
4c	H	CH_3	CH_2	72 190-92	$C_{25}H_{27}N_7S$	Brown shining crystals	66.23 (65.62)	5.03 (5.95)	20.77 (21.43)
4d	H	CH_3	NCH_2CH_3	68 166-68	$C_{26}H_{30}N_8S$	White shining flakes	62.32 (64.17)	5.66 (6.21)	21.35 (23.03)
4e	H	C_3H_7	CH_2	71 140-42	$C_{27}H_{31}N_7S$	Yellow shining powder	63.25 (66.77)	4.99 (6.43)	18.79 (20.19)
4f	H	C_3H_7	NCH_2CH_3	73 184-86	$C_{28}H_{34}N_8S$	Yellow crystals	63.24 (65.34)	7.03 (6.66)	20.62 (21.77)
4g	2,4-dinitro	H	CH_2	63 221-23	$C_{23}H_{24}N_9O_4S$	Red shining crystals	53.11 (54.02)	4.01 (4.34)	22.68 (23.63)
4h	2,4-dinitro	H	NCH_2CH_3	78 234-36	$C_{25}H_{26}N_{10}O_4S$	Red shining crystals	50.98 (53.37)	3.60 (4.66)	22.48 (24.90)
4i	2,4-dinitro	CH_3	CH_2	81 230-32	$C_{25}H_{25}N_9O_4S$	Red shining crystals	52.32 (54.83)	3.15 (4.60)	21.94 (23.02)
4j	2,4-dinitro	CH_3	NCH_2CH_3	63 205-07	$C_{26}H_{28}N_{10}O_4S$	Red shining crystals	55.25 (54.16)	4.02 (4.89)	23.42 (24.29)
4k	2,4-dinitro	C_3H_7	CH_2	75 242-44	$C_{27}H_{29}N_9O_4S$	Red shining crystals	55.36 (56.34)	4.56 (5.08)	20.34 (21.90)
4l	2,4-dinitro	C_3H_7	NCH_2CH_3	74 227-30	$C_{28}H_{32}N_{10}O_4S$	Red shining crystals	53.85 (55.62)	3.14 (5.33)	21.55 (23.16)

Solvent for recrystallization: EtOH + Dioxane

2.3.1: 4-{[(1,3-Diphenyl-1*H*-pyrazol-4-yl)methylidene]amino}-2-(piperidin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4a**).

IR (KBr) ν cm^{-1} : 2928(CH-str.); 1600(N=CH-str.); 1117 (C=S str.); ^{1}H-NMR (300MHz, solvent $CDCl_3$, δ in ppm): δ, 1.39-1.69(m, 6H, -CH_2-CH_2-CH_2-); 2.79(t, 4H, -CH_2-N-CH_2-); 5.16(s, 2H, N-CH_2); 7.47-8.10(m, 10H, Ar-H); 8.81 (s, 1H, triazole-5H); 9.00 (s, 1H, pyrazole-5H); 10.10(s, 1H, N=CH). MS m/z; 443 (M^+ + 1).

2.3.2: 4-{[(1,3-Diphenyl-1*H*-pyrazol-4-yl)methylidene]amino}-2-[(4-ethylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4b**).

IR (KBr) ν cm^{-1} : 2924(CH-str); 1604(N=CH-str.); 1115 (C=S str.). ^{1}H-NMR (300MHz); (solvent $CDCl_3$, δ in ppm): δ, 2.16(t, 3H, CH_3); 3.16(q, 2H, CH_2); 4.39-4.69(m, 8H, piperazinyl protons); 5.16(s, 2H, N-CH_2); 7.42-8.14(m, 10H, Ar-H); 8.81 (s, 1H, Triazole-5H) 9.0 (s, 1H, pyrazole-5H); 10.102(s, 1H, N=CH). MS m/z; 472 (M^+ + 1).

2.3.3: 4-{[(1,3-Diphenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-methyl-2-(piperidin-1-yl methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4c**).

IR (KBr) ν cm^{-1}: 2978(CH-str); 1612 (N=CH-str.); 1108 (C=S str.); ^{1}H-NMR (300MHz); (solvent $CDCl_3$, δ in ppm): δ, 1.39-1.69(m, 6H, -CH_2-CH_2-CH_2-); 2.79(m, 4H, -CH_2-N-CH_2-); 2.16(s, 3H, CH_3);5.16(s, 2H, N-CH_2); 7.40-8.11(m, 10H, Ar-H); 8.81 (s, 1H, triazole-5H) 9.0 (s, 1H, pyrazole-5H); 10.10(s, 1H, N=CH). MS m/z; 457(M^+ + 1).

2.3.4: 4-{[(1,3-Diphenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-methyl-2-[(4-ethylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4d**).
IR (KBr) ν cm^{-1}: 2966 (CH-str.); 1640 (N=CH-str.); 1113 (C=S str.). ^{1}H-NMR (300MHz), (solvent $CDCl_3$, δ in ppm), 2.16(t, 3H, CH_3); 2.86(s, 3H, CH_3); 3.16(q, 2H, CH_2); 4.39-4.69(m, 8H, piperazinyl protons); 5.16(s, 2H, N-CH_2); 7.43-8.14(m, 10H, Ar-H); 8.810 (s, 1H, triazole-5H); 9.0(s, 1H, pyrazole-5H); 10.102(s, 1H, N=CH). MS m/z; 472 (M^+ + 1).
2.3.5:: 4-{[(1,3-Diphenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-propyl-2-(piperidin-1-yl methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4e**).
IR (KBr) ν cm^{-1}: 2976 (Ar-H str.); 1602 (N=CH-str.); 1117 (C=S str.); ^{1}H-NMR (300MHz), (solvent $CDCl_3$, δ in ppm), 1.39-1.69(m, 6H, -CH_2-CH_2-CH_2-); 2.79(t, 4H, -CH_2-N-CH_2-); 2.11 (t, 3H, CH_3); 2.21 (m, 2H, CH_2); 2.32 (t, 2H, CH_2);5.16(s, 2H, N-CH_2); 7.41-8.18(m, 10H, Ar-H); 8.81 (s, 1H, triazole-5H); 9.0 (s, 1H, pyrazole-5H); 10.10(s, 1H, N=CH). MS m/z; 485 (M^+ + 1).
2.3.6: 4-{[(1,3-Diphenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-propyl-2-[(4-ethylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4f**).
IR (KBr) ν cm^{-1}: 2918 (CH-str.); 1549 (N=CH-str.); 1132 (C=S str.); ^{1}H-NMR (300MHz); (solvent $CDCl_3$, δ in ppm), 2.16(t, 3H, CH_3); 2.86(s, 3H, CH_3); 3.16(q, 2H, CH_2); 2.11 (t, 3H, CH_3); 2.21 (m, 2H, CH_2); 2.32 (t, 2H, CH_2); 4.39-4.69(m, 8H, piperazinyl protons); 5.16(s, 2H, N-CH_2); 7.42-8.10 (m, 10H, Ar-H); 8.81 (s, 1H, triazole-5H); 9.0 (s, 1H, pyrazole-5H); 10.10(s, 1H, N=CH). MS m/z; 514 (M^+ + 1).
2.3.7:4-{[(1-(2,4-Dinitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl)methylidene]amino}-2-(piperidin-1-yl methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4g**).
IR (KBr) ν cm^{-1}: 2974(CH-str.); 1608 (N=CH-str.); 1140 (C=S str.); ^{1}H-NMR (300MHz); (solvent $CDCl_3$, δ in ppm), 1.39-1.69(m, 6H, -CH_2-CH_2-CH_2-); 2.79(t, 4H, -CH_2-N-CH_2-); 5.16(s, 2H, N-CH_2); 7.41-8.20 (m, 8H, Ar-H); 8.810 (s, 1H, triazole-5H) 9.0 (s, 1H, pyrazole-5H); 10.10 (s, 1H, N=CH). MS m/z; 533 (M^+ + 1).
2.3.8: 4-{[(1-(2,4-Dinitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl)methylidene]amino}-2-[(4-ethylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4h**).
IR (KBr) ν cm^{-1}: 2998 (CH-str.); 1587(N=CH-str.); 1109 (C=S str.); ^{1}H-NMR (300MHz); (solvent $CDCl_3$, δ in ppm), 2.16(t, 3H, CH_3); 3.16(q, 2H, CH_2); 4.39-4.69(m, 8H, piperazinyl protons); 5.16(s, 2H, N-CH_2); 7.34-8.08 (m, 8H, Ar-H); 8.810 (s, 1H, triazole-5H); 9.0 (s, 1H, pyrazole-5H); 10.12 (s, 1H, N=CH). MS m/z; 562 (M^+ + 1).
2.3.9: 4-{[(1-(2,4-Dinitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-methyl-2-(piperidin-1-yl methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4i**).
IR (KBr) ν cm^{-1} : 2928(CH-str.); 1600 (N=CH-str.); 1127 (C=S str.); ^{1}H-NMR (300MHz); (solvent $CDCl_3$, δ in ppm), 1.39-1.69(m, 6H, -CH_2-CH_2-CH_2-); 2.79(t, 4H, -CH_2-N-CH_2-); 2.16(s, 3H, CH_3);5.16(s, 2H, N-CH_2); 7.32-7.98(m, 10H, Ar-H); 8.81 (s, 1H, triazole-5H) 9.0 (s, 1H, pyrazole-5H); 10.23 (s, 1H, N=CH). MS m/z; 457(M^+ + 1).
2.3.10: 4-{[(1-(2,4-Dinitrophenyl)-,3-phenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-methyl-2-[(4-ethylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4j**).
IR (KBr) ν cm^{-1}: 2966 (Ar-H str.); 1612 (N=CH-str.); 1117 (C=S str.); ^{1}H-NMR (300MHz); (solvent $CDCl_3$, δ in ppm), δ, 2.16(t, 3H, CH_3); 2.86(s, 3H, CH_3); 3.16(q, 2H, CH_2); 4.39-4.69(m, 8H, piperazinyl protons); 5.16(s, 2H, N-CH_2); 7.4-8.1(m, 8H, Ar-H); 8.810 (s, 1H, Triazole-5H) 9.000 (s, 1H, pyrazole-5H); 10.102(s, 1H, N=CH). MS m/z; 472 (M^+ + 1).
2.3.11:4-{[(1-(2,4-Dinitrophenyl)-3-phenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-propyl-2-(piperidin-1-yl methyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4k**).
IR (KBr) ν cm^{-1}: 2965(CH-str.); 1612 (N=CH-str.); 1115 (C=S str.), ^{1}H-NMR (300MHz); (solvent $CDCl_3$, δ in ppm), 1.39-1.69(m, 6H, -CH_2-CH_2-CH_2-); 2.79(t, 4H, -CH_2-N-CH_2-); 2.11 (t, 3H, CH_3); 2.21 (m, 2H, CH_2); 2.32 (t, 2H, CH_2);5.16(s, 2H, N-CH_2); 7.32-8.19 (m,

8H, Ar-H); 8.81 (s, 1H, triazole-5H) 9.10 (s, 1H, pyrazole-5H); 10.21 (s, 1H, N=CH). MS m/z; 575(M^+ + 1).

2.3.12:4-{[(1-(2,4-dinitrophenyl)-,3-phenyl-1*H*-pyrazol-4-yl)methylidene]amino}-5-propyl-2-[(4-ethylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**4l**).

IR (KBr) ν cm^{-1}: 2988 (CH-str.); 1598 (N=CH-str.); 1132 (C=S str.), ^{1}H-NMR (300MHz); (solvent $CDCl_3$, δ in ppm), 2.16(t, 3H, CH_3); 2.86(s, 3H, CH_3); 3.16(q, 2H, CH_2); 2.11 (t, 3H, CH_3); 2.21 (m, 2H, CH_2); 2.32 (t, 2H, CH_2); 4.39-4.69(m, 8H, piperazinyl protons); 5.16(s, 2H, N-CH_2); 7.34-8.16 (m, 8H, Ar-H); 8.81 (s, 1H, triazole-5H); 9.08 (s, 1H, pyrazole-5H); 10.18 (s, 1H, N=CH). MS m/z; 604 (M^+ + 1).

3.0:Biological activity

3.1: Antimicrobial activity.

The newly synthesized Mannich bases (**4a-l**) were screened for their *in vitro* antibacterial activity against both Gram-positive and Gram-negative bacteria. *Staphylococcus Aureus* (NCIM 2794), *Bacillus Subtilis* (NCIM 2708), *Escherichia Coli* (NCIM 2575), and *Pseudomonas Aeruginosa* (NCIM 2053) were the microorganisms employed. Furacin was used as standard. The antifungal activity was screened *in vitro* against *Candida Albicans* (NCIM 3466) and Fluconazole was the standard. The experiment was carried out by Minimum inhibitory concentration (MIC) by serial dilution method [XX]. For this, the compound whose MIC has to be determined is dissolved in serially diluted dimethylformamide. Then a standard drop of the culture prepared for the assay is added to each of the dilutions and incubated for 16-18hrs at 37^0C. MIC is the highest dilution of the compound, which shows clear fluid with no development of turbidity (**Table 3**).

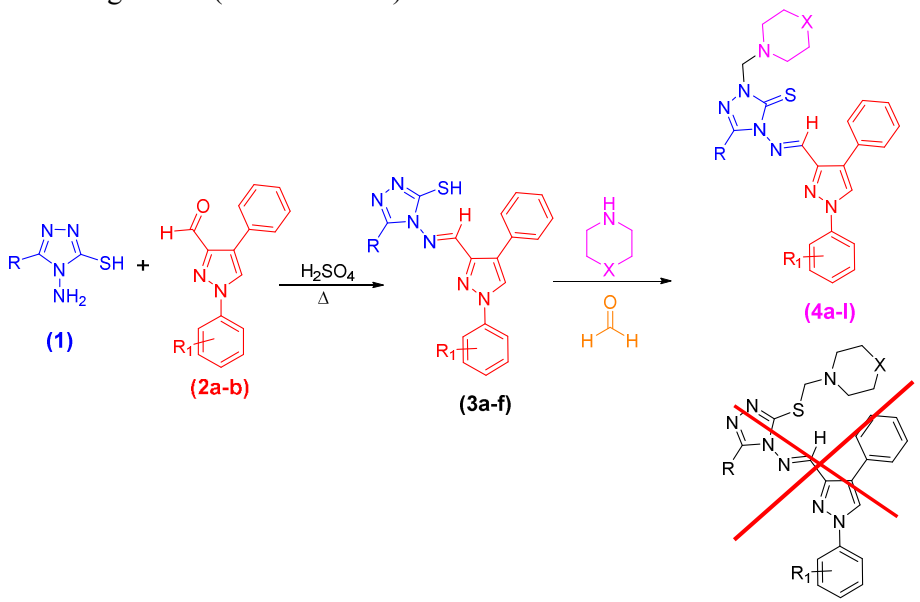
3.2: Anthelmintic activity

The anthelmintic activity studies were carried out against earthworm (Pentoscoplex corethrusus) [XXI]. Six earthworms of the approximately same size were placed in each petri dish (4" size) containing 50mL of suspension of specific concentrations at 28 ± 1ºC. Simultaneously a control having 6 worms in distilled water and tween 80 (0.5%) was kept. The drug concentrations used were 0.1 % (w/v) and 0.2% (w/v) for both standard and test. The time required for paralysis (movements stopped) and death time of the worms was noted using a stopwatch. The death time of the worms was ascertained by transferring them to a beaker containing hot water (50°C), which stimulated and induced movements if the worm was alive. The mean data obtained are given in (**Table 4)**.

4.0: Results and discussion.

3-Substituted-4-amino-5-mercapto-1,2,4-triazoles (**1**) were synthesized as per the procedure reported in the literature [XXII] and 1-aryl-3-phenyl-1H-pyrazole-4-carbaldehyde (**2)** were prepared by the Vilsmeir Haack formylation of corresponding phenylhydrazones in presence of dimethyl formamide and phosphorous oxychloride ($POCl_3$) [XXIII]. The Schiff bases (**3a-f**) were prepared by the condensation of amino mercapto triazoles (**1**) with substituted pyrazole aldehyde(**2a-b**) in presence of catalytic amount of concentrated sulphuric acid in ethanol solvent. These Schiff bases (**3a-f**), when subjected to Mannich reaction with an appropriate secondary amine in the presence of formaldehyde, gave N-Mannich derivatives (**4a-l**) (**Scheme. 1**). Although the Schiff base can exhibit thiol-thione tautomerism [III] interestingly molecule (**3a-f**) reacted through nitrogen nucleophile and resulted in the formation of N-Mannich base rather than S-Mannich base. The reaction is highly regioselective and throughout the reaction, we have observed the formation of N-Mannich bases. Newly synthesized compounds (**3a-f** and **4a-l**) were characterized by IR, ^{1}H-NMR,

mass spectral and C, H, N analysis. The characterization data of the newly synthesized compounds were given in (**Table 1** and **2**).



R= H, CH_3, C_3H_7; R_1= H, 2,4-$(NO_2)_2$; X=CH_2, N-CH_2CH_3.
Scheme 1: Regioselective synthesis of Mannich base (**4a-l**)

The structure of the newly synthesized compounds was confirmed by elemental analysis, IR, NMR and mass spectral analysis. The characterization data of Schiff bases (**3a-f**) and their Mannich bases (**4a-h**) are given in (**Table 1**) and (**Table 2**) respectively. The results of elemental analysis agree with the theoretical values within the limits of experimental error.
The Schiff bases when subjected to Mannich reaction, gave N-Mannich bases rather than S-Mannich base. The regioselectivity in the reaction was also confirmed by recording the proton NMR spectra of these Mannich bases (**4a**). In the ^{1}H-NMR spectra of these Mannich bases, the signal at δ, 5.16 is typical of the N-CH_2group. Further, the absence of any signal in the region of δ, 4.0-4.5 clearly reveals the absence of S-CH_2 group thereby indicating that the Mannich reaction has occurred through nitrogen atom yielding the N-Mannich bases.
The formation of N-Mannich base was further confirmed by recording their IR spectra. In all the cases the absorption band due to the thione (C=S) group was observed at 1113 cm^{-1}.
Further evidence for the proposed structure of Mannich bases was obtained by recording their mass spectra. In all the cases the mass spectra recorded were in accordance with the assigned molecular formulae.

4.1: Biological Activity.
4.1.1: Antimicrobial activity.
The newly synthesized Mannich (**4a-l**) bases showed moderate to good antimicrobial activity. Among tested compounds, compound (**4l)** (12.5)showed prominent bacterial inhibition against all the tested bacterial isolates. All other compounds in this series exhibited moderate to good bacterial inhibition. All the synthesized Mannich derivatives showed excellent antifungal activity compared to the standard Fluconazole (**Table 3**). The result indicates the importance of Schiff and Mannich bases embedded heterocyclic systems in antimicrobial activity.
Table 3: Antibacterial and Antifungal Activity data of Mannich Bases (**4a-l**).

Comp. No.	Antibacterial data in MIC (µg/mL)				Antifungal data in MIC (µg/mL)
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis,*	*Candida. albicans*
4a	6	6	6	3	6
4b	6	6	6	6	6
4c	6	6	6	6	6
4d	6	6	6	6	6
4e	6	6	6	6	6
4f	12.5	6	12.5	6	6
4g	12.5	12.5	6	12.5	6
4h	12.5	6	12.5	6	6
4i	6	6	6	3	6
4j	12.5	12.5	6	12.5	6
4k	12.5	6	12.5	6	6
4l	12.5	12.5	12.5	12.5	6
Furacin	12.5	12.5	12.5	12.5	-
Fluconazole	-	-	-	-	6
DMF (control)	-	-	-	-	-

4.1.2: Anthelmintic activity

All the newly synthesized Mannich (**4a-l**) bases showed moderate to excellent anthelmintic activity. From the observation, it is clear that the synthesized Mannich base carrying 2,4-dinitrophenyl and N-ethyl piperazine (**4h**) exhibited excellent anthelmintic activity compared to standard employed Albendazole. The compound (**4h**) showed minimum paralyzing time (16.44min) and (15.40min) in both the concentration (100 and 200 mg),which is lower than the paralyzing time of the standard (20.26 min and 17.40 min). The mean death time (41.36 min (100mg) and 32.42min (200mg)) also indicated synthesized Mannich bases (**4h**) showed excellent anthelmintic activity as compared to the standard and with the rest of the synthesized compounds.

Table-4: Anthelmintic activity data of Mannich Bases (**4a-l**)

Comp	**Mean paralyzing in min**		**Mean death time in min**	
	100mg	200mg	100mg	200mg
4a	18.40	16.22	44.26	36.18
4b	15.28	16.12	44.32	40.38
4c	28.58	22.26	46.26	48.38
4d	18.36	16.52	46.24	36.42
4e	18.38	16.24	46.28	41.32
4f	25.40	20.34	50.18	42.38
4g	17.26	15.12	44.48	35.26
4h	16.44	15.40	41.36	32.42
4i	22.44	19.44	44.18	42.56
4j	19.45	17.22	48.33	41.48

4k	18.46	20.43	44.58	46.27
4l	20.32	18.18	47.47	46.43
Std	**20.26**	**17.40**	**42.02**	**32.68**

Conflict of Interest
The authors declare that there is no conflict of interest.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Received on May 24, 2019.