Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(12):163-171
(http://www.derpharmachemica.com/archive.html)

Synthesis, Antimicrobial and Anthelmintic Activity Studies of Some Novel Triazole Schiff and Mannich Bases

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ABSTRACT

A series of new Mannich bases (E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-(substituted methyl)-1H-1,2,4-triazole-5(4H)-thione (6a-e) & (7a-h) were synthesized by treating (E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1H-1,2,4-triazole-5(4H)-thione (5) with various substituted primary/ secondary amines and formaldehyde. The Schiff base was obtained by treating 3-methyl-4-amino-5-mercapto-1,2,4-triazole (3) with 3,4-dimethoxybenzaldehyde (4) in the presence of acid catalyst. The triazole (3) was prepared by treating acetic acid (1) with thiocarbohydrazide (2) at reflux temperature. The newly synthesized compounds were confirmed by spectroscopic techniques and screened for their antimicrobial and anthelmintic activity.

Keywords: 1,2,4-triazole, Mannich bases, Aminomethylation, Antibacterial, Antifungal, Anthelmintic

INTRODUCTION

Antimicrobial agents are one of the most important weapons in human inventory that have assisted him for world dominance from the near extinction due to epidemic storms, wars and other traumas. Emergence of microbial resistance by the overuse and abuse of antimicrobials is posing a major threat now which has been a hot topic around the world. To overcome this two faced strategy is recommended globally, which includes; a) use of antimicrobials in a scientific manner to reduce the selective pressures for development of resistance and b) development of novel classes of antimicrobial agents.

Several high throughput screening techniques is an integral part of drug discovery and development. Library screening and biochemical assays were widely till recent years, but the failure of high-throughput biochemical assays to deliver useful hit molecules that could be developed into drugs has refocused the hit discovery process back onto whole-cell activity screening [1]. Have defined criterias for good antibiotics in their review. Successful antibiotics inhibit critical pathways in the bacterial cell, and the very best are bactericidal and they are usually molecules that can be manipulated and modified for the purpose of identifying analogues with more desirable antibacterial activities against the desired range of target pathogens, and to achieve good pharmacokinetic/pharmacodynamic properties with acceptably low levels of toxicity against mammalian cells. The most valuable antibiotics are soluble drugs, chemically stable, that can be taken orally and act systemically. This range of desirable properties places a premium on smaller molecules that in principle should be easier to modify chemically, achieve wide distribution and tissue penetration within the body and be amenable to production on an industrial scale.

Multicomponent reactions are a major part of synthetic organic chemistry with several advantages ranging from lower reaction times and temperatures to higher yields. A three-component condensation reaction of Mannich reaction involves active hydrogen containing compound, formaldehyde and a secondary amine [2]. The amino alkylation of aromatic substrates by Mannich reaction have its own importance for the synthesis and modification of biologically active compounds, similarly Schiff base derivatives of 1,2,4-triazole also have shown significant biological activity [3]. The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important drug candidates including histamine receptor blockers, cholinesterase active agents, CNS stimulants, anxiolytics, and sedatives [4]. Fluconazole, itraconazole and voriconazole (antimycotic) [5], ribavirin (antiviral), rizatriptan (antimigraine), alprazolam (anxiolytic), vorozole, letrozole and anastrozole (antitumoral) are some examples of drugs containing 1,2,4-triazole moiety [6-10].

1,2,4-triazoles were also reported to possess significant antimicrobial, analgesic, anti-inflammatory, anticancer and antioxidant properties [11-14]. Literature survey revealed that Mannich bases possess potent biological activities such as antibacterial, antifungal, anti-inflammatory, antimalarial and pesticide properties [15-18]. Some of the Mannich bases are reported to have antitubercular, antimalarial, vasorelaxing, anticancer and analgesic drugs [19-24].

Experimental section

The melting points of the compounds (6a-e) and (7a-h) were determined by an open capillary method and are uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FTIR 157 spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded ($CDCl_3$/DMSO-d_6 mixture) on 400 MHz spectrometer using Tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded in Agilent Technology LC-mass spectrometer and GC/MS spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer using argon/xenon (6kv, 10 mA). Elemental analyses (CHNS) were performed on the CHNS Elementar Vario EL III. The progress of the reaction was monitored by Thin Layer Chromatography (TLC) on silica gel plates.

General procedure for the synthesis of 3-methyl-4-amino-5-mercapto-1,2,4-triazole (3)

An equimolar mixture of glacial acetic acid (1) and thiocarbohydrazide (2) was heated on an oil bath till the contents melted. The reaction mixture was maintained at this temperature for 3 h. Then it was allowed to cool and treated with dilute sodium bicarbonate solution in order to remove any unreacted acid. The solid was filtered, washed with water, dried and recrystallized from ethanol to obtain the pure triazole (3) (Scheme 1A).

General procedure for the synthesis of (E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1H-1,2,4-triazole-5(4H)-thione (5)

To a solution of 3,4-dimethoxybenzaldehyde (4) (10 mmol) in methanol was added an equimolar amount of the amino mercaptotriazole (3) and a few drops of concentrated sulfuric acid as a catalyst at room temperature. The reaction mixture was heated to reflux temperature for 8 h. The precipitated compound was filtered and recrystallized from hot methanol (Scheme 1B).

General procedure for the synthesis of (E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-(substituted methyl)-1H-1,2,4-triazole-5(4H)-thione (6a-e) & (7a-h)

A mixture of Schiff bases (5) (10 mmol), formaldehyde (40%, 1.5 ml) and appropriate primary/secondary-amines (10 mmol) in ethanol medium was stirred at room temperature for 12 h. The precipitated solid was filtered under suction, washed with cold ethanol and recrystallized from hot ethanol.

4-amino-3-methyl-1H-1,2,4-triazole-5(4H)-thione (3)

IR (KBr) cm^{-1}: 3374 (N–H), 2839 (C–H), 1602 (C=N), 1272 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.46 (s, 3H, CH_3), 10.10 (s, 1H, SH/NH), 10.29 (s, 1H, N=CH); LC-MS (m/z, %): 131.17.

(E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1H-1,2,4-triazole-5(4H)-thione (5)

IR(KBr) cm^{-1}: 3374 (N–H), 2839 (C–H), 1602 (C=N), 1272 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.46 (s, 3H, CH_3), 3.96 (s, 6H, $(OCH_3)_2$), 6.93-6.95 (d, 1H, J=8 Hz), 7.36-7.38 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.48 (d, 1H, J=2Hz), 10.10 (s, 1H, SH/NH), 10.29 (s, 1H, N=CH); LC-MS (m/z, %): 276.50.

(E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-(morpholinomethyl)-1H-1,2,4-triazole-5(4H)-thione (6a)

IR (KBr) cm^{-1}: 3072 (aromatic C–H), 2933 (C-H), 1600 (C=N), 1274 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.45 (s, 3H, CH_3), 2.82 (t, 4H, marpholine-$(CH_2)_2$), 3.70 (t, 4H, marpholine-$(CH_2)_2$), 3.96 (s, 6H, $(OCH_3)_2$), 5.09 (s, 2H, N–CH_2–N), 6.93-6.95 (d, 1H, 3,4-dimethoxy phenyl, J=6.8Hz), 7.35-7.38 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.49-7.49 (d,1H, 3,4-dimethoxy phenyl, J=2Hz), 10.08 (s, 1H, N=CH), ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 11.73 (CH_3 group of triazole), 51.91, 51.90, 56.61 and 56.70 (marpholine), 67.43, 67.50 (2C atom of dimethoxy), 69.62 (-CH_2), 109.62, 111.39, 125.34, 125.91, 148.36 and 150.14 (3,4-dimethoxy phenyl), 153.65 (-C=N of schiff base), 162.93 (-C=N of triazole), 187.03 (-C=S); GCMS (m/z, %) 378.4 (M^+).

(E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-(piperidin-1-ylmethyl)-1H-1,2,4-triazole-5(4H)-thione (6b)

IR(KBr) cm^{-1}: 3080 (aromatic C–H), 2933 (C-H), 1597 (C=N), 1265 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 1.58 (m, 6H, piperidine), 2.45 (s, 3H, CH_3), 2.77 (t, 4H, piperidine-$(CH_2)_3$), 3.95 (d, 6H, $(OCH_3)_2$), 5.09 (s, 2H, N–CH_2–N), 6.93 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.35-7.38 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.49-7.49 (d, 1H, 3,4-dimethoxy phenyl, J=2Hz), 10.11 (s, 1H, N=CH), ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 10.43 (CH_3 group of triazole), 24.01, 30.48, 30.36, 49.77, 49.94 (piperidine C), 55.31, 55.43 (2C atom of dimethoxy), 68.14 (-CH_2), 95.97, 108.45, 110.15, 124.02, 148.87, 151.45 (3,4-dimethoxy phenyl), 161.57 (-C=N of schiff base), 162.09 (-C=N of triazole), 175.13 (-C=S); GCMS (m/z,%) 376.8(M^+).

(E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-(pyrrolidin-1-ylmethyl)-1H-1,2,4-triazole-5(4H)-thione (6c)

IR(KBr) cm^{-1}: 3073 (aromatic C–H), 2928 (C-H), 1602 (C=N), 1261 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.42 (s, 3H, CH_3), 3.95 (d, 6H, $(OCH_3)_2$), 5.09 (s, 2H, N–CH_2–N), 6.93 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.14-7.31 (m, 4H, pyrolidine), 7.35-7.38 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.49 (d,1H, 3,4-dimethoxy phenyl, J=2Hz), 10.11 (s,1H, N=CH), ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 10.38 (CH_3 group of triazole), 48.78, 48.92, 55.22, 55.92 (pyrolidine C), 58.09, 58.48 (2C atom of dimethoxy), 68.14 (-CH_2), 108.38, 110.32, 124.27, 124.91, 148.82 and 151.13 (3,4-dimethoxy phenyl), 161.74 (-C=N of schiff base), 162.04 (-C=N of triazole), 175.02 (-C=S); GCMS (m/z, %) 362.36 (M^+)

(E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-((4-methylpiperazin-1-yl)methyl)-1H-1,2,4-triazole-5(4H)-thione (6d)

IR(KBr) cm^{-1}: 3062 (aromatic C–H), 2946 (C-H), 1599 (C=N), 1242 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.42 (s, 3H, CH_3), 2.51 (s, 3H, N-CH_3), 2.92 (t, 4H, piperazine), 3.09 (t, 4H, piperazine), 3.91 (s, 6H, $(OCH_3)_2$), 5.07 (s, 2H, N–CH_2–N), 6.91-6.92 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.35-7.37 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.49-7.49 (d,1H, 3,4-dimethoxy phenyl, J=2Hz), 10.09 (s, 1H, N=CH), ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.69 (CH_3 group of triazole), 40.03 (-N-CH_3), 51.51, 51.54, 56.21 and 56.72 (piperazine C), 67.49, 69.58 (2C atom of dimethoxy), 71.11 (-CH_2),109.67, 111.37, 125.24, 125.78, 150.16 and 150.29 (3,4-dimethoxy phenyl), 153.67 (-C=N of schiff base),

162.97 (-C=N of triazole), 187.07 (-C=S); GC/MS (m/z, %) 391.6 (M^+).

(E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-(piperazin-1-ylmethyl)-1H-1,2,4-triazole-5(4H)-thione (6e)

IR(KBr) cm^{-1}: 3450 (N-H), 3072 (aromatic C–H), 2933 (C-H), 1600 (C=N), 1271 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 1.39 (m, 1H, N-H), 1.60 (t, 4H, piperazine), 2.45 (s, 3H, CH_3), 2.77 (t, 4H, piperazine), 3.95 (s, 6H, $(OCH_3)_2$, J=0.8Hz), 5.09 (s, 2H, N–CH_2–N), 6.93 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.35-7.38 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.49 (d,1H, 3,4-dimethoxy phenyl, J=2Hz), 10.11 (s, 1H, N=CH), ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.14 (CH_3 group of triazole), 42.85, 42.93, 51.89, and 51.91 (piperazine C), 56.57, 56.61 (2C of dimethoxy phenyl), 69.94 (-CH_2), 109.04, 110.78, 124.61,125.39, 147.46, 149.49 (3,4-dimethoxy phenyl), 152.94 (-C=N of schiff base), 162.08 (-C=N of triazole), 187.07 (-C=S); GC/MS (m/z, %) 377.2(M^+).

(E)-1-(((4-bromophenyl)amino)methyl)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1H-1,2,4-triazole-5(4H)-thione (7a)

IR (KBr) cm^{-1}: 3373 (N-H), 3078 (aromatic C–H), 2933 (C-H), 1593 (C=N), 1274 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.33 (s, 3H, CH_3), 3.82 (d, 6H, $(OCH_3)_2$), 5.44 (d, 2H, N–CH_2–N, J=7.2Hz), 6.86-6.87 (d, 2H, 3,4-dimethoxy phenyl, J=2Hz), 6.88 (d, 2H, 4-Br-phenyl, J=2Hz), 7.10-7.12 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.22-7.36 (d, 2H, 4-Br-phenyl, J=2Hz), 7.43-7.36 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.48-7.49 (d, 1H, 3,4-dimethoxy phenyl, J=2Hz), 10.08 (s, 1H, N=CH), ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.73 (CH_3 group of triazole), 56.61, 56.70 (2C of dimethoxy phenyl), 70.04 (-CH_2), 109.62, 111.39, 125.34, 125.91, 148.36 and 150.14 (3,4-dimethoxy phenyl), 111.21,111.29, 117.61, 136.89, 126.34 and 126.42 (C atom of 4-bromo phenyl), 153.65 (-C=N of schiff base), 162.93 (-C=N of triazole), 187.03 (-C=S); GC/MS (m/z, %) 364.4 (M^++2).

(E)-4-((3,4-dimethoxybenzylidene)amino)-1-(((4-ethoxyphenyl)amino)methyl)-3-methyl-1H-1,2,4-triazole-5(4H)-thione (7b)

IR(KBr) cm^{-1}: 3375 (N-H), 3071 (aromatic C–H), 2939 (C-H), 1589 (C=N), 1280 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.34 (s, 3H, CH_3), 2.39 (q, 2H, CH_2 of ethyl, J=4.2 Hz) 3.81 (d, 6H, $(OCH_3)_2$), 5.42 (d, 2H, N–CH_2–N, J=7.2Hz), 6.89-6.91 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.31-7.32 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.34-7.35 (d,1H, 3,4-dimethoxy phenyl, J=2Hz), 7.52-7.65 (m, 4H, 2-ethoxyphenyl), 10.08 (s,1H, N=CH),^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.72 (CH_3 group of triazole), 12.72, 25.36 (ethoxy C) 56.62, 56.75 (2C of dimethoxy phenyl), 70.04 (-CH_2), 109.62, 111.39, 125.34, 125.91, 141.34, 148.36 (3,4-dimethoxy phenyl), 120.62, 120.98, 121.28, 121.32, 140.35 and 150.14 (C atoms os ethoxy phenyl), 153.65 (-C=N of triazole), 162.93 (-C=N of schiff base), 187.03 (-C=S); GC/MS (m/z, %) 383.3 (M^+).

(E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-((pyrazin-2-ylaminomethyl)-1H-1,2,4-triazole-5(4H)-thione (7c)

IR (KBr) cm^{-1}: 3378 (N-H), 3081 (aromatic C–H), 2943 (C-H), 1599 (C=N), 1278 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.30 (s, 3H, CH_3), 3.79 (d, 6H, $(OCH_3)_2$), 5.39 (d, 2H, N–CH_2–N, J=7.2Hz), 6.91-6.92 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.33-7.34 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.36-7.37 (d, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.40 (d, 1H, pyrazine, J=2.4 Hz), 7.41 (d, 1H, pyrazines, J=2.4 Hz), 7.43 (s, 1H, pyrazine), 10.06 (s, 1H, N=CH), ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.68 (CH_3 group of triazole), 56.21, 56.36(2C of dimethoxy phenyl), 71.29 (-CH_2), 109.52, 110.36, 123.21, 126.62, 142.02 and 150.14 (3,4-dimethoxy phenyl), 136.21, 138.12, 155.72, 159.52 (pyrazine C atoms), 153.23 (-C=N of triazole), 162.76 (-C=N of schiff base), 185.21 (-C=S); GC/MS (m/z, %) 386.1 (M^+).

(E)-4-((3,4-dimethoxybenzylidene)amino)-1-(((3,4-dimethoxyphenyl)amino)methyl)-3-methyl-1H-1,2,4-triazole-5(4H)-thione (7d)

IR (KBr) cm^{-1}: 3063 (aromatic C–H), 2912 (C-H), 1608 (C=N), 1276 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.4 (s, 3H, CH_3), 3.90 (s, 6H, $(OCH_3)_2$, 5.07 (s, 2H, N–CH_2–N), 6.92-6.94 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 6.96-6.98 (d, 1H, 3,4-dimethoxy phenyl, J=8.2Hz), 7.34-7.35 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.36-7.37 (d, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.39-7.40 (d, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.42-7.43 (d, 1H, 3,4-dimethoxy phenyl, J=2Hz), 10.04 (s,1H, N=CH), ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.68 (CH_3 group of triazole), 56.42, 56.65 (2C of dimethoxy phenyl), 72.26 (-CH_2), 102.36, 107.33, 109.52, 111.63, 113.23, 126.11, 128.80,139.59, 140.38, 142.71, 148.51 and 150.23 (C atoms of dimethoxy phenyl rings), 153.65(-C=N of triazole), 161.78(-C=N of schiff base), 185.22 (-C=S); GC/MS (m/z, %) 344.9(M^+).

(E)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-(((2,4,5-trichlorophenyl)amino)methyl)-1H-1,2,4-triazole-5(4H)-thione (7e)

IR (KBr) cm^{-1}: 3063 (aromatic C–H), 2912 (C-H), 1608 (C=N), 1269 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.39 (s,3H, CH_3), 3.88 (s, 6H, $(OCH_3)_2$), 5.06 (s, 2H, N–CH_2–N), 6.89-6.92 (d, 1H, 3,4-dimethoxy phenyl, *J*=8.4Hz), 7.32-7.33 (dd,1H, 3,4-dimethoxy phenyl, J=2Hz), 7.35-7.36 (d, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.69 (s, 1H, 2,4,5-trichlorophenyl), 7.92 (s, 1H, 2,4,5-trichlorophenyl), 10.08 (s, 1H, N=CH), ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.75 (CH_3 group of triazole), 56.43, 56.92 (2C of dimethoxy phenyl), 70.91 (-CH_2), 109.69, 111.23, 118.71, 123.46, 125.24, 126.11, 131.72, 132.29, 132.64, 147.24, 149.21, 153. 21, 158.46 (-C=N of triazole), 161.81 (-C=N of schiff base), 187.81 (-C=S); GCMS (m/z, %) 488.6 (M^++2).

(E)-1-(((4-chlorophenyl)amino)methyl)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1H-1,2,4-triazole-5(4H)-thione (7f)

IR (KBr) cm^{-1}: 3074 (aromatic C–H), 2926 (C-H), 1601 (C=N), 1263 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.38 (s, 3H, CH_3), 3.81 (s, 6H, $(OCH_3)_2$), 5.01 (s, 2H, N–CH_2–N), 6.91-6.93 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.41-7.42 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.44-7.45(d,1H,3,4-dimethoxy phenyl, J=2Hz, 7.54 (d, 2H, 4-chlorophenyl, J=8 Hz), 7.68 (d, 2H, 4-chlorophenyl, J=8 Hz), 10.07 (s, 1H, N=CH), ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.69 (CH_3 group of triazole), 55.19, 55.62 (2C of dimethoxy phenyl), 70.89(-CH_2), 109.72, 111.88, 125.24, 126.23, 133.84,149.50 (3,4-dimethoxy phenyl), 112.11, 112.41, 119.32, 138.71, 126.34 and 126.42 (C atom of 4-bromo phenyl 154.96 (-C=N of triazole), 158.91 (-C=N of schiff base), 187.81 (-C=S); GC/MS (m/z, %) 419.7 (M^++2).

(E)-1-(((3-chloro-4-fluorophenyl)amino)methyl)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1H-1,2,4-triazole-5(4H)-thione (7g)

IR (KBr)cm^{-1}: 3079 (aromatic C–H), 2914 (C-H), 1612 (C=N), 1271 (C=S); ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 2.31 (s, 3H, CH_3), 3.78 (s, 6H, $(OCH_3)_2$), 5.06 (s, 2H, N–CH_2–N), 7.13-7.15 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.22-7.23 (dd, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.27-7.28 (d, 1H, 3,4-dimethoxyphenyl, J=2Hz), 7.37-7.46 (m, 3H, 3-chloro-4-fluorophenyl), 10.06 (s, 1H, N=CH); ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.61 (CH_3 group of triazole), 55.10, 55.25 (2C of dimethoxy phenyl), 70.66 (-CH_2), 109.11, 111.31, 123.46, 129.24, 149.50 and 153.09 (3,4-dimethoxy phenyl), 121.34, 121.85, 123.46, 123.89, 146.31 and 148.12 (3-Cl,4-F-phenyl C), 154.31 (-C=N of triazole), 158.91 (-C=N of schiff base), 187.04 (-C=S); GC/MS (m/z, %) 438.82 (M^++2)

5(E)-4-((3,4-dimethoxybenzylidene)amino)-1-((diphenylamino)methyl)-3-methyl-1H-1,2,4-triazole-5(4H)-thione (7h)

IR (KBr) cm^{-1}: 3083 (aromatic C–H), 2921 (C-H),160 (C=N), 1273(C=S); ^{1}H-NMR(400 MHz, $CDCl_3$, δ ppm): 2.30 (s,3H, CH_3), 3.76 (s, 6H, $(OCH_3)_2$), 5.01 (s, 2H, N–CH_2–N), 6.91-6.93 (d, 1H, 3,4-dimethoxy phenyl, J=8.4Hz), 7.36-7.37 (d, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.38-7.39 (d, 1H, 3,4-dimethoxy phenyl, J=2Hz), 7.24-7.43 (m, 5H, phenyl), 7.51-7.80 (m, 5H, phenyl), 10.03 (s, 1H, N=CH); ^{13}C-NMR (400 MHz, $CDCl_3$, δ ppm): 11.51 (CH_3 group of triazole), 56.58, 56.64 (2C of dimethoxy phenyl), 70.91 (-CH_2), 109.11, 111.41, 123.31, 133.42, 148.33 and 150.18 (3,4-dimethoxy phenyl), 121.03, 121.14, 121.85, 121.91, 132.52, 133.60, 133.72, 133.81, 133.97, 135.17, 135.48, 151.74 and 151.82 (C atoms of diphenyl C), 153.69 (-C=N of triazole), 158.19 (-C=N of schiff base), 187.09 (-C=S); GC/MS (m/z, %) 460.51 (M^+).

Pharmacology

The novel 4-[(*Z*)-(3,4-disubstituted) amino]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a-e) and (7a-h) were synthesized and evaluated for antimicrobial and anthelmintic activity studies.

Antimicrobial activity

All the newly synthesized compounds were screened for their *in vitro* antibacterial activity against the Gram-positive *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (ATCC 6633) and Gram-negative *Escherichia coli* (NCIM 2931), *Klebsiella pneumoniae* (NCIM 2957) procured from National Chemical Laboratory, Pune, India.

Antibacterial assay was carried out by disc diffusion method [25]. For *in vitro* antibacterial activity, 200 µl of overnight grown culture of each organism was dispensed into 20 ml of sterile nutrient broth and incubated for 4-5 h at 37°C to standardize the culture to 10^{-5} CFU/ml. For this, 0.1 ml (10^{-5} CFU/ml) of 24 h old bacterial culture was placed on Muller Hinton agar medium and spread throughout the plate by spread plate technique.

The synthesized (*E*)-1-Aryl-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione derivatives dissolved in Dimethyl Sulphoxide (DMSO) were loaded to the sterile discs (250 µg concentration, 6 mm diameter disc) purchased from HIMEDIA laboratories, individually and aseptically before screening for antibacterial activity. Antibacterial activity was recorded by measuring the diameter of zone of inhibition. Streptomycin was used as positive and DMSO as negative standard against bacterial strains respectively.

Antifungal activity

Antifungal activities of all the synthesized compounds were tested by potato dextrose agar well diffusion method. The microorganisms used were *Aspergillus niger, Cladospora* spp., *Candida albicans* and *Trichoderma viride*. The test was run in triplicates. Pure cultures of the organisms were inoculated onto potato dextrose agar incubated for 72 h, 37°C.

The antifungal property of the each compound was carried out by potato dextrose agar well diffusion method in order to measure the zone of inhibition Hasan et al. The compounds were dissolved in DMSO to get a concentration of 10 mg/ml (1.0 in DMSO). Fluconazole (5 mg/ml) was used as reference standard and solvent control was also maintained throughout the experiment. The screening was initiated by inoculating the test fungi on to nutrient broth under incubation temperature of 37°C for 72 h. From the broth, lawn of each test fungi was made with the help of sterile cotton swabs on nutrient agar plates. Well of 0.5 cm in diameter was punched on the plate with the help of sterile cork borer. The well was filled with varied concentrations of the compounds and the experiment was carried out in triplicate. Plates were incubated for 72 h at 37°C after loading the extracts. The plates were then observed for clear zone formation around the well. Antifungal activities were expressed in millimeter [26].

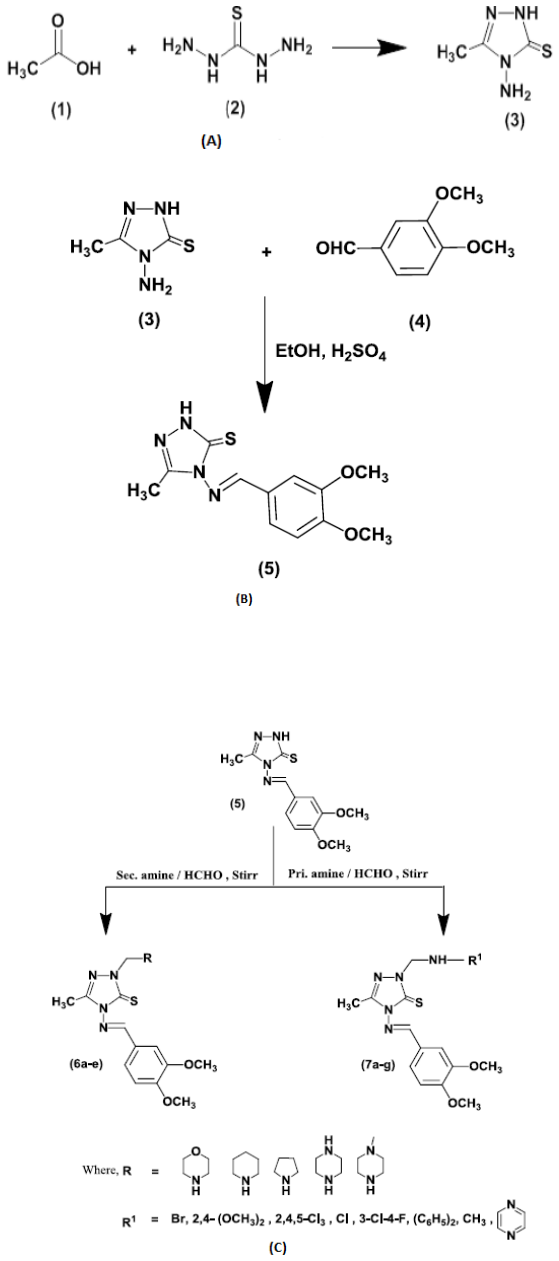
Anthelmintic activity

Indian adult earthworms (*Eudrilus eugeniae)* collected from earthworm rearing center, Davangere (Karnataka), were washed with normal saline to remove all fecal matter and used for the anthelmintic study. The earthworms of 3-5cm in length and 0.1-0.2 cm in width were used. The anthelmintic activity was evaluated on Indian adult earthworms due to its anatomical and physiological resemblance with the intestinal round worm parasites of human being. They were divided into 12 groups of 6 earthworms each of approximately equal size were released into 25 ml of desired dextrose solution. The experiment was carried out in one petri plate for each group. To study anthelmintic property each petri plate was treated with one of the following, (1% normal saline), albendazole (10 mg/ml) or 2% of each compounds [27]. Observations were made for the time taken to cause paralysis and death time of the individual worms. Death was concluded when the worms lost their motility followed with fading away of their body colors. The experiment was carried out in one petri plate for each group.

RESULTS AND DISCUSSION

Chemistry

The novel series of 4-[(*Z*)-(3,4-disubstituted) amino]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a-e) and (7a-h) were synthesized according to the procedure reported in the literature [1]. The equimolar ratio of glacial acetic acid (1) and thiocarbohydrazide (2) were heated for 3 h, followed by treatment with sodium bicarbonate solution (Scheme 1A). The resultant 3-methyl-4-amino-5-mercapto-1,2,4-triazole (3) was then treated with 3,4-dimethoxybenzaldehyde (4) with catalytic amount of concentrated sulfuric acid to give (*E*)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1*H*-1,2,4-triazole-5(4*H*)-thione(5) (Scheme 1B). Then the Schiff base (5) obtained was stirred with equimolar ratio of appropriate primary/secondary amines to yield the series of novel (*E*)-4-((3,4-dimethoxybenzylidene)amino)-3-methyl-1-(substitutedmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thion e (6a-e) and (7a-h) (Scheme 1C). The structures of newly synthesized compounds (6a-e) and (7a-h) were confirmed by spectral and elemental analysis (Table 1). Its data has been incorporated in the experimental section.



Scheme 1: (A-C) Reaction scheme

Pharmacological screening

The antibacterial activity of the newly synthesized compounds (6a-e) and (7a-h) was evaluated by zone inhibition method by using streptomycin as positive control. The antifungal activity was tested by potato dextrose agar well diffusion method using fluconazole (5%) as positive control. Anthelmintic activity was evaluated on Indian adult earthworms due to its anatomical and physiological resemblance with the intestinal round worm parasites of human being using albendozole as positive control.

Antibacterial activity

The antibacterial activity of the newly synthesized target compounds (6a-e) and (7a-h) was evaluated by zone inhibition method taking Streptomycin as a standard drug (Table 2) (Figure 1). Among the various novel Schiff and Mannich bases, all the compounds exhibited more or less antibacterial activity except (7a) and (7f). Compounds which showed the antibacterial activity possess differential potency against bacterial strains with reference to their growth inhibition activity. (6a) and (6b) showed the activity against all the bacterial strains; but the observed activity at a lower range except (6b) which showed the good activity against *K. pneumonia* as indicated by zone of inhibition 16 ± 0.78 mm. Considering the zone of inhibition value more than 12 mm, following compounds can be considered to possess substantial antibacterial activity: (6b) (against *S. aureus* and *K. pneumonia*); (6e) (against *B. subtilis*); (7d) (against *K. pneumonia*); (7e) (against *K. pneumonia*); (7g) (against *S. aureus*) and (7h) (against *B. subtilis*). The compounds such as (6b), (7d) and (7g) which showed the maximum activity in terms of zone of inhibition can be considered as drug candidates against diseases caused by *K. pneumonia, S. aureus*, respectively. Among the compounds, (6b) with bromo substitution on phenyl ring for which all the bacterial strains showed the sensitivity (>10 mm) can be regarded as a broad-spectrum antibacterial compound, and further studies may be taken up for their therapeutic values.

Antifungal activity

Antifungal activity was tested by potato dextrose agar well diffusion method comparative with fluconazole standard drug of 5% (Table 3) (Figure 2). Compounds 6c, 6d, 6e, 7b, 7c, 7e and 7f showed the inhibitory effect all fungal strains tested. 6a, 7e exhibited activity against *C. albicans* and *A. niger*. 7a and 7g was active against *Cladospora spp* and *A. niger*, 7d inhibited only *A. niger*.

Table 1: Characterization data of 4-[(*Z*)-(3,4-disubstituted) amino]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a-e) and (7a-h)

Compound	R	Molecular formula (Molecular weight)	Yield (%)	m.p. (°C)	%Composition, found (Calcd)		
					C	H	N
6a	Morpholine	$C_{17}H_{23}N_5O_3S$ (377)	79	165-168	54.09 (54.04)	6.14 (6.17)	18.55 (18.52)
6b	Piperidine	$C_{18}H_{25}N_5O_2S$ (375)	72	177-180	57.58 (57.56)	6.71 (6.69)	18.65 (18.67)
6c	Pyrolidine	$C_{17}H_{23}N_5O_2S$ (361)	87	204-207	56.49 (56.43)	6.41 (6.44)	19.38 (19.36)
6d	N-methyl piperazine	$C_{18}H_{26}N_6O_2S$ (461)	80	240-243	55.36 (55.34)	6.71 (6.73)	21.52 (21.54)
6e	piperazine	$C_{17}H_{24}N_6O_2S$	78	159-162	54.20 (54.23)	6.39 (6.43)	22.33 (22.32)
7a	4-Br- C_6H_5	$C_{19}H_{20}BrN_5O_2S$ (390)	90	223-226	49.36 (49.34)	4.36 (4.33)	15.15 (15.14)
7b	4-(OC_2H_5) - C_6H_5 p-phenetidine	$C_{21}H_{25}N_5O_3S$ (427)	85	210-213	59 (59.1)	5.89 (5.87)	16.38 (16.37)
7c	pyrazine	$C_{17}H_{19}N_7O_2S$ (385)	92	216-219	52.97 (52.96)	4.97 (4.96)	25.44 (25.43)
7d	3,5-(OCH_3)–C_6H_4	$C_{21}H_{25}N_5O_4S$ (443)	84	256-259	56.87 (56.86)	5.68 (5.67)	15.79 (15.78)
7e	2,4,5-$(Cl)_3$ – C_6H_3	$C_{19}H_{18}Cl_3N_5O_2S$ (486)	93	184-187	46.88 (46.87)	3.73 (3.72)	14.39 (14.38)
7f	4-Cl-C_6H_5	$C_{19}H_{18}ClN_5O_2S$ (417)	84	191-194	54.61 (54.59)	4.82 (4.79)	16.76 (16.78)
7g	3-Cl-4-F-C_6H_4	$C_{19}H_{19}ClFN_5O_2S$ (435)	96	192-195	52.38 (52.36)	4.39 (4.38)	16.07 (16.05)
7h	$(C_6H_5)_2$	$C_{25}H_{25}N_5O_2S$ (459)	83	162-165	65.34 (65.21)	5.48 (5.39)	15.24 (15.21)

Table 2: Antibacterial activity (Zone of inhibition) of 4-[(*Z*)-(3, 4-disubstituted)amino]-5- methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a-e) and (7a- h) and streptomycin is a reference standard

	Zone of inhibition (mm)			
Samples	***Staphylococcus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Klebsiella pneumoniae***
6a	10 ± 0.00	11 ± 0.00	10.13 ± 0.20	9 ± 0.00
6b	12.9 ± 0.1	10.06 ± 0.11	11.53 ± 0.50	16 ± 0.00
6c	12 ± 0.00	9 ± 0.00	-	9.76 ± 0.25
6d	-	9 ± 0.00	-	9 ± 0.00
6e	10 ± 0.00	12.16 ± 0.15	-	10 ± 0.00
7a	-	-	-	-
7b	11.96 ± 0.05	-	-	9 ± 0.00
7c	8 ± 0.00	10 ± 0.00	-	-
7d	10 ± 0.00	11.16 ± 0.15	-	15.2 ± 0.34
7e	8 ± 0.00	10.26 ± 0.05	-	13.93 ± 0.11
7f	-	-	-	-
7g	16.16 ± 0.28	10.1 ± 0.17	-	10.26 ± 0.25
7h	8 ± 0.03	12.23 ± 0.05	-	11.72 ± 0.00
Streptomycin	24.3 ± 0.30	22.16 ± 0.37	18.73 ± 0.64	20.6 ± 0.52

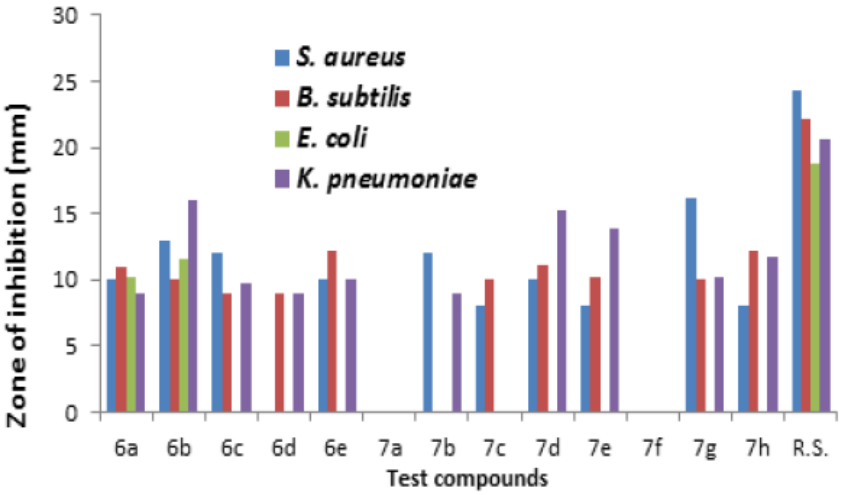


Figure 1: Antibacterial activity of 4-[(*Z*)-(3, 4-disubstituted) amino]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a-e) and (7a-h) and streptomycin is a reference standard

The newly synthesized Schiff and Mannich bases (6a-e) and (7a-g) exhibited the antifungal activity; all of them were found to be positive but with differential potency Table 3. Among the compounds, some of them showed a good activity against one or the other fungal strains, which include (6a) against *Cladospora* spp., (15.16 ± 0.28 mm); (6c) against *C. albicans* (19.96 ± 0.45 mm); (6d) and (7e) against *C. albicans* (16.16 ± 0.28 mm) and (16.06 ± 0.11 mm), (7c) against *C. albicans* (18.91 ± 0.16 mm) and (7f) against *C. albicans* (15.78 ± 0.30 mm). Thus, these compounds can be regarded as the derivatives with potential antifungal activity against a specific fungal species. Considering the zone of inhibition values between 12 and 15 mm, the compounds such as (6a), (7b) and (7f) were found to possess the growth inhibition activity against *C. albicans* at moderate level. Among the compounds, (6c) with pyrolidine and (7c) with pyrazine substitutions which exhibited the maximum activity (19.96 ± 0.45 mm and 18.91 ± 0.16 mm, respectively) can be regarded as drug candidates against the disease caused by *C. albicans* and further studies may be taken up.

Table 3: Antifungal activity (Zone of inhibition) of 4-[(*Z*)-(3, 4-disubstituted)amino]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a-e) and (7a-g) and fluconazole is a reference standard

Zone of inhibition (mm)				
Samples	***Candida albicans***	***Cladospora* spp.**	***Aspergillus niger***	***Aspergillus fumigates***
6a	13.1 ± 0.36	15.16 ± 0.28	-	10.23 ± 0.25
6b	9.1 ± 0.10	-	10.06 ± 0.30	-
6c	19.96 ± 0.45	9 ± 0.00	10.16 ± 0.28	-
6d	16.16 ± 0.28	10.1 ± 0.17	10.26 ± 0.25	-
6e	16.06 ± 0.11	11.33 ± 0.30	-	-
7a	-	10.06 ± 0.11	9.23 ± 0.40	8.86 ± 0.23
7b	15.12 ± 0.8	8.00 ± 0.23	9.09 ± 0.45	-
7c	18.91 ± 0.16	9.19 ± 0.23	10.01 ± 0.10	-
7d	-	-	8.02 ± 0.20	7.19 ± 0.29
7e	15.78 ± 0.30	-	7.00 ± 0.45	7.91 ± 0.35
7f	13.09 ± 0.10	7.98 ± 0.21	7.45 ± 0.40	-
7g	-	6.75 ± 0.25	7.45 ± 0.20	-
Fluconazole	26.3 ± 0.30	22.46 ± 0.37	20.73 ± 0.64	23.6 ± 0.52

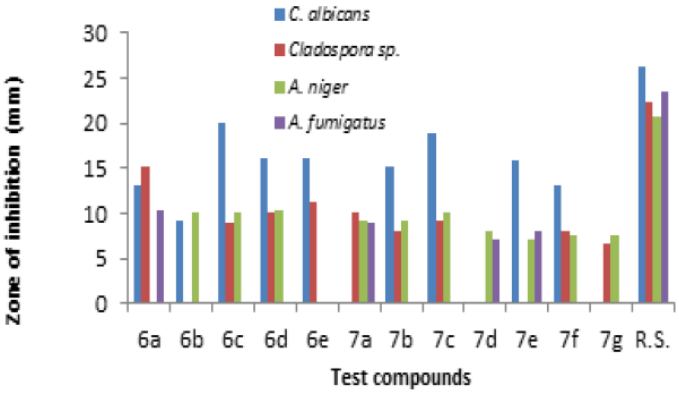


Figure 2: Antifungal activity of 4-[(*Z*)-(3, 4-disubstituted) amino]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a-e) and (7a-h) and reference standard (R.S.)

Table 4: Anthelmintic activity of 4-[(*Z*)-(3, 4-disubstituted)amino]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a-e) and (7a-g) and albendazole is a reference standard

Samples	Paralysis time in min	Death time in min
6a	12.4	14.30
6b	11.22	13.43
6c	10.52	13.07
6d	9.42	11.58
6e	7.59	10.23
7a	13.24	15.46
7b	9.35	12.05
7c	14.47	16.16
7d	10.37	13.18
7e	15.25	18.36
7f	14.58	16.28
7g	9.32	12.20
Albendazole	10.65	14.52

Anthelmintic activity

Table 4 and Figure 3 present the data obtained for anthelmintic activity of the novel derivatives. All the tested compounds showed the anthelmintic activity in terms of paralysis followed by death at the tested dose. Compounds (6c), (6d) and (6e), (7b) and (7g) exhibited the higher sensitivity to earthworm as indicated by the minimum time taken for paralysis and killing. Among the novel derivatives (6e), which showed its response, killing the earthworm by 10 min can be considered as a potent anthelmintic agent. The reason for its higher efficacy is not known. However, compounds (6c), (6d), (6e), (7a) and (7g) with substitutions pyrolidine, *N*-methyl piperazine, piperazine, 4-Br-C_6H_4 and 3-Cl-4-F-C_6H_4 groups may be having some toxic or binding affinity to one or the other molecule leading to death of the representative helminthic species.

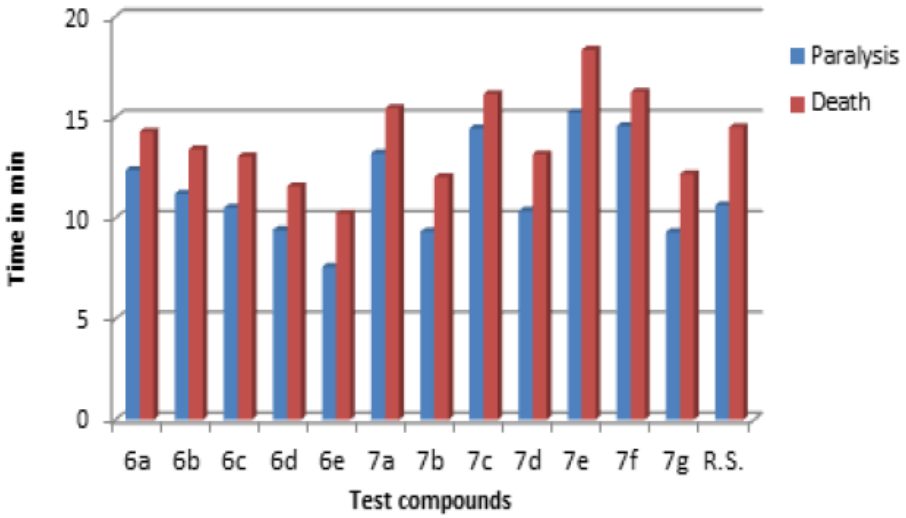


Figure 3: Antheliminitic activity of 4-[(*Z*)-(3, 4-disubstituted) amino]-5-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a-e) and (7a-h) and albendazole is a reference standard

CONCLUSION

A series of novel Mannich bases, namely E)-4-((3, 4-dimethoxybenzylidene)amino)-3-methyl-1-(substituted methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (6a-e) and (7a-h) were synthesized and characterized by NMR, mass spectrometry and IR studies. These possess an array of interesting chemical and physicochemical properties, as well as a variety of biological activities. In this regard we synthesized Schiff and Mannich derivatives possessing 1,2,4-triazole moiety with the objective of developing molecules possessing better antimicrobial and anthelmintic property. All the newly synthesized compounds were screened for their antimicrobial activity evaluated by zone inhibition method and anthelmintic activity.

Structure activity analysis

Current guidelines recommend tests for identifying Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) values as the most accurate preliminary screening technique for identifying potential antimicrobial agents. Since this method has certain drawbacks like need for large number of tests (around 10 tubes per compound for high throughput screenings which are usually with more than 10 novel compounds which makes it 100 tubes per strain and considering the need for a minimum of two gram positive and two gram negative strains total number of tubes would be 400). The well diffusion or disc diffusion method which are accepted for routine laboratory antibiotic sensitivity tests are not recommended for testing novel candidates since the diffusion properties of the compound are not standardized and could interfere with the results. We propose that disc diffusion tests and well diffusion tests is quite useful for the preliminary screening test for selection of candidates for MIC or MBC tests.

6c (pyrolidine) and 6d (N-methyl piperazine) inhibited all the fungal strains tested indicating broad antifungal activity. 6a (marpholine) and 6e (piperazine) inhibited *Cladospora spp* and *C. albicans*, 6b (piperidine) inhibited *Cladospora spp* and *A. niger*.

7a (4-Br-C_6H_5) and 7f (4-Cl-C_6H_5) substituted compounds didn't show antibacterial property against any of the four bacterial strains tested. 7c (pyrazine) inhibited *S. aureus* and *B. subtilis* i.e., possess selective inhibition of Gram-positive organisms. 7e (2,4,5-$(Cl)_3$- C_6H_3), 7h (($C_6H_5)_2$), 7d (3,5-(OCH_3) -C_6H_4), 7b (4-(OC_2H_5)–C_6H_5), 7g (3-Cl-4-F-C_6H_4) inhibited all organisms tested except *E. coli* (Figure 1).

Antifungal activity study reveals that the compound 7b (4-(OC_2H_5)-C_6H_5), 7c (pyrazine) and 7f (4-Cl-C_6H_5) inhibited *C. albicans, Cladospora spp*., and *A. niger*. 7a (4-Br-C_6H_5), 7g (3-Cl-4-F-C_6H_4) inhibited *Cladospora spp*., and *A. niger* but was not effective against *C. albicans*. 7e (2,4,5-$(Cl)_3$-C_6H_3) inhibited *C. albicans* and *A. niger* but was not effective against *Cladospora spp*., 7d (3,5-(OCH_3) -C_6H_4) inhibited *A. niger* but was not effective against *C. albicans* and *Cladospora* spp. (Figure 2).

Anthelmintic activity study revealed that the compounds 6c, 6d, 6e, 7b and 7g having pyrolidine, N-methyl piperazine, piperazine, p-phenetidine and 3-Cl-4-Fl phenyl substitution exhibited excellent activity compared to the standard drug albendazole (Figure 3). Remaining compounds 6a, 6b, 7a, 7c, 7d, 7e and 7f having marpholine, piperidine, 4-Br, pyrazine, 3,5-(OCH_3), 2,4,5-$(Cl)_3$ derivatives showed comparable activity against the standard drug.

The antimicrobial development program based on these novel derivatives looks promising with several different activity profiles like broad antibacterial, broad antifungal, selective gram positive, antifungal or antibacterial activities on structural modifications. This indicates the vast possibilities of these compounds and demands further detailed antimicrobial analysis with MIC, MBC test and *in vitro/in vivo* toxicity tests.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the UGC, Govt. of India, New Delhi, for granting RGNF fellowship. We are grateful to Sapala Organics Pvt. Ltd. (Hyderabad), ^{1}H-NMR, IISER (Pune) for ^{13}C-NMR and USIC, Mangalore University for providing FT-IR reported herein.

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