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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Some novel Schiff bases of [1,2,4]triazole bearing haloarene moiety—synthesis and evaluation of antituberculosis properties and neutrophil function test

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Abstract A series of novel Schiff bases of [1,2,4]triazole-bearing haloarene moiety (**5a–l**) were synthesized from 4-amino-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (**3**) and were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR, LC-Mass spectra and elemental analyses and screened for in vitro antituberculosis properties and for neutrophil function test, where they exhibited moderate neutrophil functions and antituberculosis properties. Compounds (**5a**), (**5c**), (**5f**), (**5h**) and (**5i**) were moderately sensitive to antituberculosis activity, while (**5a**), (**5b**), (**5g**) and (**5h**) were sensitive for neutrophil functions in the series. The acute oral toxicity studies on the Swiss albino mice according to the OECD/OCDE guidelines 423 confirmed that the compounds were slightly toxic and hence they may be considered as drug candidates for microbial pathogens. The antituberculosis studies by both ZOI and MIC tests confirmed that the novel derivatives are moderately sensitive towards *Mycobacterium tuberculosis* (H37RV) strain.

Keywords [1,2,4]triazole · Schiff bases · Haloarene moiety · Antituberculosis studies · Neutrophil function test

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Introduction

The compounds with c group (–**CH=N**–) are popularly known as the Schiff bases (March, 1985). They are synthesized by the condensation of primary amines and active carbonyl groups. In general, a Schiff base is a nitrogen analogue of an aldehyde or ketone in which the **>C=O** group is replaced by >**C=N**–R group. Schiff bases have drawn the attention of researchers and have surfaced to prominence due to ease in synthesis and possession of important pharmacological activities. Schiff bases derived from different heterocyclic compounds have been found to possess antibacterial, antifungal, anticancer and antiviral (Holla *et al.,* 1998; Yadav and Singh, 2001; Vinci *et al.,* 2003; Ashok *et al.,* 2007), anticonvulsant (Chaubey and Pandeya, 2012), antiproliferative (Bayrak *et al.,* 2009), herbicidal (Holla *et al.,* 2000) and antituberculosis activity (Fokes *et al.,* 2005). In addition to all these, they play vital role in inorganic chemistry as ligands, e.g.: copper (II) complex of the Schiff base salicylaldoxime, salen (a common tetra-dentate ligand) and Jacobson's catalyst (Molina and Mederos, 2003).

In addition to the observation on Schiff bases, it is observed that numerous research articles have reported of [1,2,4]triazoles as possessing excellent antibacterial, antifungal and anticancer (Holla *et al.,* 2006), antitubercular (Walczak *et al.,* 2004), analgesic and anti-inflammatory properties (Amir and Shikha, 2004). Moreover, [1,2,4]triazole core is incorporated in wide variety of therapeutically interesting molecules to transform them into better drugs. Some of the present day drugs with triazole nucleus include ribavirin (antiviral agent), rizatriptan (antimigraine agent), alprazolam (anxiolytic agent), fluconazole, itraconazole (antifungal agent) (Karthikeyan *et al.,* 2006), etc.

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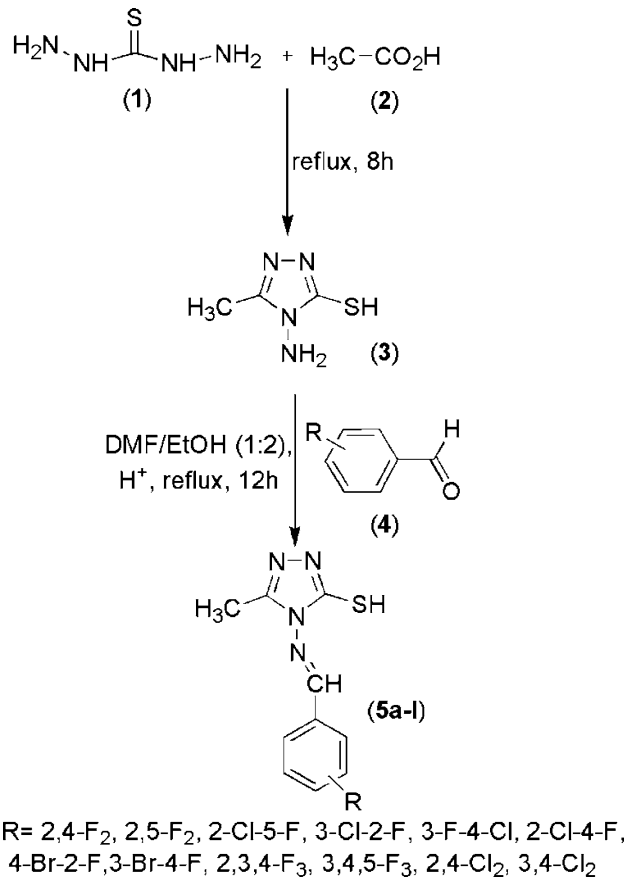
Gathering the facts together, it was decided to synthesize novel Schiff bases bearing [1,2,4]triazole and haloarene moiety. Earlier, a number of biologically active Schiff bases have been reported from our laboratory; hence, to continue the legacy of synthesizing novel Schiff bases, 4-amino-3-methyl-5-mercapto-4*H*-[1,2,4]triazole was chosen as the starting material. The synthesis, results and biological activities of the novel Schiff bases (**5a–l**) are discussed herein.

Experimental section

Melting points of the novel Schiff bases, namely 4-[(benzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (**5a–l**) were determined in an open capillary tubes and are uncorrected. The purity of synthesized compounds was checked by TLC observing a single spot on Merck silica gel 60 F_{254}-coated alumina plates. The structures of novel Schiff bases (**5a–l**) were confirmed by spectral studies. The FT-IR spectra (cm^{-1}) were recorded on a Thermo Nicolet Avatar 370 Infrared spectrophotometer in KBr pellets. The 400 MHz ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer using DMSO-d_6 solvent and TMS as the internal standard. All the chemical shift values are reported in δ-scale downfield from TMS. The Mass spectra were recorded on PerkinElmer 018444-Y Triple Quadruple LC/MS Spectrometer, and the elemental analyses were carried out on an Elementar Vario EL III analyzer.



Scheme 1 Preparation of novel Schiff bases (**5a–l**)

Procedure for the preparation of 4-amino-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (3) (Reid and Heindel, 1976)

The synthesis of 4-amino-3-methyl-5-mercapto-4*H*-[1,2,4]-triazole (**3**) was done according to the well-described procedure in the literature (Reid and Heindel, 1976). A mixture of thiocarbohydrazide (**1**) (1eq) was fused with an excess of acetic acid (**2**) at reflux temperature for 8 h. The progress of the reaction and the purity of the compound were checked by TLC. The reaction mixture was cooled and poured into crushed ice. The solid separated was collected by filtration and recrystallized from ethanol to yield needle-shaped crystals. The melting point was noted which was in conformity with the literature value (m. p. 202–204 °C, yield 55 %) (CAS No. 20939-15-5) (Scheme 1).

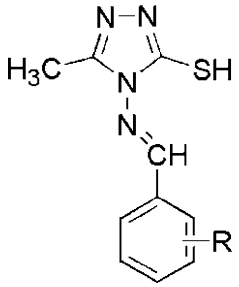
Procedure for the preparation of novel Schiff bases: 4-[(benzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5a–l) (Karthikeyan *et al.*, 2006)

A mixture of 4-amino-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (**3**) (0.01 mol) and substituted aromatic aldehyde (**4**) (0.01 mol) was refluxed for 12 h in DMF and ethanol solvent (1:2) and a catalytic amount of Conc. H_2SO_4 acid. The progress of the reaction was monitored by TLC. The reaction mixture was poured into crushed ice. The solid mass obtained was filtered, washed with excess of water, dried and recrystallized from ethanol and DMF mixture to yield the title compound. Melting points of the novel Schiff bases (**5a–l**) were noted (Scheme 1). The characterization data are given in Table 1.

The spectroscopic data

4-Amino-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (3) IR (KBr, cm^{-1}): 3342, 3270 (asymmetric and symmetric stretch, $–NH_2$), 2942 (>C–H stretch, $–CH_3$ of 1,2,4-triazole), 2580 (–SH stretch), 1579, 1507 (>C=N–), 1476 (>N–N< stretch) and 1214 (>C–S– stretch). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.24 (3H, s, $–CH_3$ of 1,2,4-triazole), 5.46 (2H, s, $–NH_2$ of 1,2,4-triazole) and 13.38 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.35 ($–CH_3$, 1,2,4-triazole), 149.12 (>C–CH_3, 1,2,4-triazole) and 165.01 (>C–SH, 1,2,4-triazole). LC-Mass, [M^++1], (m/z): 131.00. Anal. Cald for $C_3H_6N_4S$: C, 27.68; H, 4.65; N,

Table 1 Characterization data of Schiff bases (**5a–l**)



Compound	R	Mol. formula (mol. wt)	m. p. (°C) (yield %)	% Composition found (Cald)		
				C	H	N
5a	2,4-F_2	$C_{10}H_8F_2N_4S$ (254.29)	178–180 (74)	47.23 (47.24)	3.19 (3.17)	22.02 (22.04)
5b	4-Br-2-F	$C_{10}H_8BrFN_4S$ (314.25)	214–216 (66)	38.10 (38.11)	2.57 (2.56)	17.77 (17.78)
5c	2,5-F_2	$C_{10}H_8F_2N_4S$ (254.29)	212–214 (72)	47.23 (47.24)	3.18 (3.17)	22.03 (22.04)
5d	3-Br-4-F	$C_{10}H_8BrFN_4S$ (314.25)	188–190 (68)	38.10 (38.11)	2.57 (2.56)	17.77 (17.78)
5e	2-Cl-5-F	$C_{10}H_8ClFN_4S$ (270.26)	224–226 (65)	44.35 (44.37)	2.99 (2.98)	20.69 (20.70)
5f	4-Cl-3-F	$C_{10}H_8ClFN_4S$ (270.26)	204–206 (69)	44.36 (44.37)	2.99 (2.98)	20.69 (20.70)
5g	3,4,5-F_3	$C_{10}H_7F_3N_4S$ (272.24)	176–178 (68)	44.11 (44.12)	2.60 (2.59)	20.57 (20.58)
5h	2,3,4-F_3	$C_{10}H_7F_3N_4S$ (272.24)	184–186 (72)	44.10 (44.12)	2.60 (2.59)	20.58 (20.58)
5i	3-Cl-2-F	$C_{10}H_8ClFN_4S$ (270.26)	214–216 (70)	44.36 (44.37)	2.99 (2.98)	20.69 (20.70)
5j	2-Cl-4-F	$C_{10}H_8ClFN_4S$ (270.26)	220–222 (68)	44.36 (44.37)	2.99 (2.98)	20.69 (20.70)
5k	2,4-Cl_2	$C_{10}H_8Cl_2N_4S$ (286.26)	200–202 (75)	41.81 (41.82)	2.82 (2.81)	19.51 (19.51)
5l	3,4-Cl_2	$C_{10}H_8Cl_2N_4S$ (286.26)	230–232 (73)	41.81 (41.82)	2.82 (2.81)	19.50 (19.51)

43.04. Found: C, 27.67; H, 4.66; N, 43.03. m. p. 202–205 °C; yield: 55 %.

4-[(2,4-Difluorobenzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5a) IR (KBr, cm^{-1}): 3070 (>C–H stretch, 2,4-difluorophenyl moiety), 2936 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2652 (–SH stretch), 1594, 1502, 1382 (>C=N–, >C=C<), 1428 (>N–N< stretch), 1273 (>C–S– stretch), 1095 and 965 (C–F, 2,4-difluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.37 (3H, s, –CH_3 of 1,2,4-triazole), 7.27–7.32 (1H, m, 2,4-difluorophenyl moiety), 7.45–7.51 (1H, m, 2,4-difluorophenyl moiety), 8.12–8.18 (1H, m, 2,4-difluorophenyl moiety), 10.46 (1H, s, benzylidene proton, –N=CH–Ar), 13.77 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.57 (–CH_3 of 1,2,4-triazole), 148.64 (C–CH_3 of 1,2,4-triazole), 116.82, 116.92, 129.02, 129.12, 153.63 and 160.74 (6C atoms, 2,4-difluorophenyl moiety), 163.42 (>C–S–, 1,2,4-triazole) and 163.69 (C atom, benzylidene, –N=CH–Ar). LC-Mass, [M^++1], (m/z): 255.06 and [M^++3], (m/z): 257.05.

4-[(4-Bromo-2-fluorobenzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5b) IR (KBr, cm^{-1}): 3065 (>C–H stretch, 4-bromo-2-fluorophenyl moiety), 2946 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2577 (–SH stretch), 1588, 1491, 1342 (>C=N–, >C=C<), 1409 (>N–N< stretch), 1285 (>C–S– stretch), 1021 and 748 (C–F and C–Br of 4-bromo-2-fluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.38 (3H, s, –CH_3 of 1,2,4-triazole), 7.61 (1H, d, J = 8.4 Hz, 4-bromo-2-fluorophenyl moiety), 7.78 (1H, t, 4-bromo-2-fluorophenyl moiety), 8.01 (1H, t, 4-bromo-2-fluorophenyl moiety), 10.56 (1H, s, benzylidene proton, –N=CH–Ar), 13.79 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.63 (–CH_3 of 1,2,4-triazole), 148. 80 (C–CH_3 of 1,2,4-triazole), 119.65, 120.01, 126.64, 128.54, 128.66 and 152.99 (6C atoms, 4-bromo-2-fluorophenyl moiety), 159.97 (>C–S–, 1,2,4-triazole) and 162.54 (C atom, benzylidene, –N=CH–Ar). LC-Mass, [M^++1], (m/z): 314.10/315.90 (Br-79 and Br-81, ratio 1:1 due to isotopic abundance). [M^++3], (m/z): 316.60.

4-[(2,5-Difluorobenzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5c) IR (KBr, cm^{-1}): 3072 (>C–H stretch, 2,5-difluorophenyl moiety), 2940 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2581 (–SH stretch), 1583, 1502, 1383 (>C=N–, >C=C<), 1431 (>N–

N< stretch), 1275 (>C–S– stretch), 1093 and 990 (C–F, 2,5-difluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.39 (3H, s, –CH_3 of 1,2,4-triazole), 7.47–7.56 (2H, m, 2,5-difluorophenyl moiety), 7.82–7.87 (1H, m, 2,5-difluorophenyl moiety), 10.64 (1H, s, benzylidene proton, –N=CH–Ar), 13.80 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.61 (–CH_3 of 1,2,4-triazole), 148. 96 (C–CH_3 of 1,2,4-triazole), 118.24, 118.56, 121.13, 121.47, 152.36 and 156.69 (6C atoms, 2,5-difluorophenyl moiety), 159.59 (>C–S–, 1,2,4-triazole) and 161.22 (C atom, benzylidene, –N=CH–Ar). LC-Mass, [M^++1], (m/z): 255.06. [M^++3], (m/z): 257.05.

4-[(3-Bromo-4-fluorobenzylidene)amino]-3-methyl-5-mercapto-[1,2,4]triazole (5d) IR (KBr, cm^{-1}): 3063 (>C–H stretch, 3-bromo-4-fluorophenyl moiety), 2941 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2565 (–SH stretch), 1591, 1495, 1356 (>C=N–, >C=C<), 1407 (>N–N< stretch), 1281 (>C–S– stretch), 1018 and 738 (C–F and C–Br, 3-bromo-4-fluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.37 (3H, s, –CH_3 of 1,2,4-triazole), 7.39 (1H, t, J = 6.4 Hz, 3-bromo-4-fluorophenyl moiety), 8), 7.63 (1H, dd, J = 2.4 Hz, 3-bromo-4-fluorophenyl moiety), 8.18 (1H, dd, J = 6.4 Hz 3-bromo-4-fluorophenyl moiety), 10.61 (1H, s, benzylidene proton, –N = CH–Ar), 13.68 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.58 (-CH_3 of 1,2,4-triazole), 148. 85 (C–CH_3 of 1,2,4-triazole), 120.65, 121.71, 126.84, 129.68, 130.76 and 151.97 (6C atoms, 3-bromo-4-fluorophenyl moiety), 159.59 (>C–S–, 1,2,4-triazole) and 163.51 (C atom, benzylidene, –N=CH–Ar). [M^++1], (m/z): 314.10/315.90 (Br-79 and Br-81, ratio 1:1 due to isotopic abundance). [M^++3], (m/z): 316.60.

4-[(2-Chloro-5-fluorobenzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5e) IR (KBr, cm^{-1}): 3073 (>C–H stretch, 2-chloro-5-fluorophenyl moiety), 2920 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2581 (–SH stretch), 1588, 1499, 1399 (>C=N–, >C=C<), 1461 (>N–N< stretch), 1270 (>C–S– stretch), 1091 and 824 (C–F and C–Cl, 2-chloro-5-fluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.39 (3H, s, –CH_3 of 1,2,4-triazole), 7.39–7.44 (2H, m, 2-chloro-5-fluorophenyl moiety), 7.66 (1H, dd, J = 2.4 Hz, 2-chloro-5-fluorophenyl moiety), 8.23 (1H, dd, J = 6.4 Hz, 2-chloro-5-fluorophenyl moiety), 10.81 (1H, s, benzylidene proton, –N = CH–Ar), 13.79 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.65 (–CH_3 of 1,2,4-triazole), 148.91 (C–CH_3 of 1,2,4-triazole), 115.85, 117.70, 126.92, 129.63, 136.27 and 155.28 (6C atoms, 2-chloro-5-fluorophenyl moiety), 159.13 (>C–S–, 1,2,4-triazole) and 165.33 (C atom, benzylidene, –N=CH–Ar). LC-Mass, [M^++1], (m/z): 271.20, [M^++3], (m/z): 273.10/275.10 (Cl-35 and Cl-37, ratio 3:1 due to isotopic abundance). [M^++5], (m/z): 279.10.

4-[(4-Chloro-3-fluorobenzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5f) IR (KBr, cm^{-1}): 3063 (>C–H stretch, 4-chloro-3-fluorophenyl moiety), 2954 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2576 (–SH stretch), 1586, 1487, 1366 (>C=N–, >C=C<), 1461 (>N–N< stretch), 1267 (>C–S– stretch), 1018 and 778 (C–F and C–Cl, 4-chloro-3-fluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.38 (3H, s, –CH_3 of 1,2,4-triazole), 7.61 (1H, t, J = 7.2 Hz, 4-chloro-3-fluorophenyl moiety), 7.79 (1H, t, J = 2.4 Hz, 4-chloro-3-fluorophenyl moiety), 8.03 (1H, dd, J = 6.4 Hz, 4-chloro-3-fluorophenyl moiety), 10.59 (1H, s, benzylidene proton, –N = CH–Ar), 13.68 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.59 (-CH_3 of 1,2,4-triazole), 149. 80 (C-CH_3 of 1,2,4-triazole), 119.69, 123.15, 127.44, 130.74, 132.56 and 153.99 (6C atoms, 4-chloro-3-fluorophenyl moiety), 159.77 (>C–S–, 1,2,4-triazole) and 164.14 (C atom, benzylidene, –N=CH–Ar,). LC-Mass, [M^++1], (m/z): 271.10, [M^++3], (m/z): 272.90/274.90 (Cl-35 and Cl-37, ratio 3:1 due to isotopic abundance). [M^++5], (m/z): 279.10.

3-Methyl-4-[(3,4,5-trifluorobenzylidene)amino]-5-mercapto-4*H*-[1,2,4]triazole (5g) IR (KBr, cm^{-1}): 3067 (>C–H stretch, 3,4,5-trifluorophenyl moiety), 2948 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2575 (–SH stretch, 1,2,4-triazole), 1594, 1497, 1362 (>C=N–, >C=C<), 1470 (>N–N< stretch), 1268 (>C–S– stretch), 1063, 1019 and 978 (C–F, 3,4,5-trifluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.38 (3H, s, –CH_3 of 1,2,4-triazole), 7.67–7.72 (1H, m, 3,4,5-trifluorophenyl moiety), 8.23–8.29 (1H, m, 3,4,5-trifluorophenyl moiety), 10.58 (1H, s, benzylidene proton, –N=CH–Ar), 13.74 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.56 (–CH_3 of 1,2,4-triazole), 149.81 (C–CH_3 of 1,2,4-triazole), 121.21, 123.68, 133.49, 136.27, 153.93 and 160.41 (6C atoms, 2,3,4-trifluorophenyl moiety), 162.92 (>C–S–, 1,2,4-triazole) and 164.89 (C atom, benzylidene, –N=CH–Ar). LC-Mass, [M^++1], (m/z): 273.31 and [M^++3], (m/z): 275.40.

3-Methyl-4-[(2,3,4-trifluorobenzylidene)amino]-5-mercapto-4*H*-[1,2,4]triazole (5h) IR (KBr, cm^{-1}): 3068 (>C–H stretch, 2,3,4-trifluorophenyl moiety), 2945 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2574 (–SH stretch, 1,2,4-triazole), 1592, 1498, 1372 (>C=N–, >C=C<), 1468 (>N–N< stretch), 1271 (>C–S– stretch), 1075, 1021 and 985 (C–F, 2,3,4-trifluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.39 (3H, s, –CH_3 of 1,2,4-triazole), 7.28–7.33 (1H, m, 2,3,4-trifluorophenyl moiety), 7.47–7.53 (1H, m, 2,3,4-trifluorophenyl moiety), 10.51 (1H, s, benzylidene proton, –N=CH–Ar), 13.79 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.51 (–CH_3 of 1,2,4-triazole), 149.78 (C–CH_3 of 1,2,4-triazole), 121.32, 123.72, 133.52, 136.48, 154.73 and 161.71 (6C atoms, 2,3,4-trifluorophenyl moiety), 163.72 (>C–S–, 1,2,4-

triazole) and 165.39 (C atom, benzylidene, –N=CH–Ar). LC-Mass, [M^++1], (m/z): 273.30 and [M^++3], (m/z): 275.40.

4-[(3-Chloro-2-fluorobenzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5i) IR (KBr, cm^{-1}): 3073 (>C–H stretch, 3-chloro-2-fluorophenyl moiety), 2934 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2583 (–SH stretch, 1,2,4-triazole), 1592, 1504, 1380 (>C=N–, >C=C<), 1453 (>N–N< stretch), 1278 (>C–S– stretch), 1021 and 790 (C–F and C–Cl, 3-chloro-4-fluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.38 (3H, s, –CH_3 of 1,2,4-triazole), 7.41 (1H, t, J = 8 Hz, 3-chloro-2-fluorophenyl moiety), 7.82–7.86 (1H, m, 3-chloro-2-fluorophenyl moiety), 8.02–8.06 (1H, m, 3-chloro-2-fluorophenyl moiety), 10.62 (1H, s, benzylidene proton, –N = CH–Ar), 13.81 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.62 (–CH_3 of 1,2,4-triazole), 149.71 (C–CH_3 of 1,2,4-triazole), 120.78, 121.97, 126.11, 134.33, 152.04 and 153.21 (6C atoms, 3-chloro-2-fluorophenyl moiety), 158.05 (>C–S–, 1,2,4-triazole) and 161.22 (C atom, benzylidene –N=CH–Ar). LC-Mass, [M^++1], (m/z): 271.20, [M^++3], (m/z): 273.10/275.10 (Cl-35 and Cl-37, ratio 3:1 due to isotopic abundance). [M^++5], (m/z): 279.10.

4-[(2-Chloro-4-fluorobenzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5j) IR (KBr, cm^{-1}): 3061 (>C–H stretch, 2-chloro-4-fluorophenyl moiety), 2936 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2579 (–SH stretch, 1,2,4-triazole), 1595, 1501, 1402 (>C=N–, >C=C<), 1430 (>N–N< stretch), 1274 (>C–S– stretch), 1019 and 811 (C–F and C–Cl, 2-chloro-4-fluorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.39 (3H, s, –CH_3 of 1,2,4-triazole), 7.38–7.43 (1H, m, 2-chloro-4-fluorophenyl moiety), 7.64 (1H, dd, J = 2.4 Hz, 2-chloro-4-fluorophenyl moiety), 8.22 (1H, dd, J = 6.4 Hz 2-chloro-4-fluorophenyl moiety), 10.80 (1H, s, benzylidene proton, –N = CH–Ar), 13.79 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.62 (–CH_3 of 1,2,4-triazole), 148.92 (C–CH_3 of 1,2,4-triazole), 115.84, 117.65, 126.90, 129.61, 136.27 and 159.10 (6C atoms, 2-chloro-4-fluorophenyl moiety), 162.80 (>C–S–, 1,2,4-triazole) and 165.32 (C atom, benzylidene, –N=CH–Ar). LC-Mass, [M^++1], (m/z): 271.10, [M^++2], (m/z): 272.10/272.70 (Cl-35 and Cl-37, ratio 3:1 due to isotopic abundance). [M^++5], (m/z): 275.10.

4-[(2,4-Dichlorobenzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5k) (CAS No. 4666-44-93-9).

IR (KBr, cm^{-1}): 3058 (>C–H stretch, 2,4-dichlorophenyl moiety), 2924 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2582 (–SH stretch, 1,2,4-triazole), 1584, 1500, 1381 (>C=N–, >C=C<), 1469 (>N–N< stretch), 1277 (>C–S– stretch), 795 and 732 (C–Cl, 2,4-dichlorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.39 (3H, s, –CH_3 of triazole), 7.60 (1H, dd, J = 2 Hz, 2,4-dichlorophenyl moiety), 7.82 (1H, d, J = 2 Hz, 2,4-dichlorophenyl moiety), 8.16 (1H, d, J = 8.4 Hz, 2,4-dichlorophenyl moiety), 10.89 (1H, s, benzylidene proton, –N = CH–Ar), 13.80 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.65 (–CH_3 of 1,2,4- triazole), 137.54 (C–CH_3 of 1,2,4-triazole), 118.97, 128.25, 128.68, 129.19, 129.72 and 135.74 (6C atoms, 2,4-dichlorophenyl moiety), 154.53 (>C–S–, 1,2,4-triazole) and 161.17 (C atom, benzylidene, –N = CH–Ar). LC-Mass, [M^+], (m/z): 286.60, [M^++1], (m/z): 287.20/287.80 (Cl-35 and Cl-37, ratio 3:1 due to isotopic abundance). [M^++2], (m/z): 288.80/290.60. Anal. Cald for $C_{10}H_8Cl_2N_4S$: C, 41.82; H, 2.81; N, 19.51. Found: C, 41.81; H, 2.82; N, 19.51. m. p. 200–202 °C; yield: 75 %.

4-[(3,4-Dichlorobenzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (5l) (CAS No. 4666-44-95-1).

IR (KBr, cm^{-1}): 3056 (>C–H stretch, 3,4-dichlorophenyl moiety), 2934 (>C–H stretch, –CH_3 attached to 1,2,4-triazole), 2580 (–SH stretch, 1,2,4-triazole) 1578, 1504, 1375 (>C=N–, >C=C<), 1467 (>N–N< stretch), 1273 (>C–S– stretch), 793 and 736 (C–Cl, 3,4-dichlorophenyl moiety). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.38 (3H, s, –CH_3 of triazole), 7.19 (1H, d, J = 7.6 Hz, 3,4-dichlorophenyl moiety), 7.42 (1H, s, 3,4-dichlorophenyl moiety), 7.50 (1H, d, J = 7.6 Hz, 3,4-dichlorophenyl moiety), 10.88 (1H, s, benzylidene proton, –N = CH–Ar), 13.79 (1H, s, –SH of 1,2,4-triazole). ^{13}C-NMR (δ ppm): 10.67 (–CH_3 of 1,2,4-triazole), 137.27 (C–CH_3 of 1,2,4-triazole), 119.25, 128.63, 128.97, 129.53, 130.32 and 135.87 (6C atoms, 3,4-dichlorophenyl moiety), 153.93 (>C–S–, 1,2,4-triazole) and 160.87 (C atom, benzylidene, –N=CH–Ar). LC-Mass, [M^+], (m/z): 286.60, [M^++1], (m/z): 287.20/287.80 (Cl-35 and Cl-37, ratio 3:1 due to isotopic abundance). [M^++2], (m/z): 288.80/290.60. Anal. Cald for $C_{10}H_8Cl_2N_4S$: C, 41.82; H, 2.81; N, 19.51. Found: C, 41.81; H, 2.82; N, 19.50. m. p. 230–232 °C; yield: 73 %.

Pharmacology

The novel Schiff bases (**5a–l**) were synthesized with ease and in excellent yields, confirmed by the spectroscopic data and evaluated for in vitro antituberculosis activity studies using disc diffusion method (ZOI test) and microplate Alamar Blue assay (MABA) method (MIC test). They were also subjected to the test of various components of neutrophil functions, namely neutrophil chemotaxis, NBT test, phagocytosis and microbicidal (candidacial) assay test according to the simple and well-established procedure.

Antituberculosis activity

All the newly synthesized Schiff bases (**5a–l**) were evaluated for in vitro antituberculosis activity by disc diffusion method (ZOI test) (Isenberg, 1992 and Bauer *et al.,* 1966) and microplate Alamar Blue assay (MABA) method (MIC test) (Lourenco *et al.,* 2007) according to the standard procedure. Three drugs, namely pyrazinamide, ciprofloxacin and streptomycin, were the reference standards for the comparison in both the methods (ZOI and MIC tests).

Disc diffusion method (ZOI test) (Isenberg, 1992 and Bauer et al., 1966)

Ten microlitres (10 mg/mL) of the drug (test compounds/standards) was loaded over Whatman filter paper no. 1 discs of diameter 5 mm and dried. Discs were placed in a dish plated with 4-week-old bacteria culture *Mycobacterium tuberculosis* (H37RV) strain on 7H11 agar media supplemented with OADC (growth supplement) and incubated at 37 °C for 4 weeks for bacterial growth. Zone of inhibition was measured using Digital Caliper (Mitutoyo, Japan). Experiments were performed in triplicates, and the standard error was calculated.

Microplate Alamar Blue dye assay method (MIC test) (Lourenco et al., 2007)

All the synthesized Mannich bases were screened for in vitro antituberculosis activity against *M. tuberculosis* (H37RV) strain by microplate Alamar Blue assay method (MABA) to determine the minimum inhibitory concentration (MIC test). This methodology is non-toxic and uses a thermally stable reagent and shows a good correlation with proportional and BACTEC radiometric method.

Two hundred microlitres of the sterile deionized water was added to all the outer perimeter wells of sterile 96-well plate to minimize evaporation of the medium in the test wells during incubation. One hundred microlitres of the Middlebrook 7H9 broth was added to the 96-well plate. Serial dilution of the compounds was made directly on the plate. The final drug concentrations tested were 100–0.2 μg/mL. The plates were covered and sealed with parafilm and incubated at 37 °C for 5 days. Then, 25 μL of freshly prepared 1:1 mixture of Alamar Blue dye reagent and 10 % Tween-80 was added to the plate and incubated for 24 h. The change in the colour (i.e. blue to pink) was scored as bacterial growth. The no change in blue colour was interpreted as no growth in bacteria. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration which prevented the colour change from blue to pink.

Neutrophil function test

Neutrophils are major contributors to the host defence against invading microorganisms (especially bacteria and fungi) at the various body sites including oral cavity. Advance studies have shown that there is an impairment of neutrophil functions in aggressive and chronic form periodontitis, and hence the concept of hyperactive or primed neutrophil has dawned which is highlighting the destructive aspect of neutrophil mediated tissue damage and bone resorption through the release of cytokines, especially in aggressive periodontitis. The new concept regarding neutrophil has opened up new avenues of research for the diagnosis and treatment of aggressive as well as chronic periodontitis (Lord, 1989; Matzner, 1987; Boqomolski-Yahalom and Matzner, 1995; Nathan, 2006).

Neutrophils are found in good numbers in circulation system of gingival/periodontal tissue. In normal circumstances, in the absence of infection, neutrophils roll along the vascular endothelium, transiently attaching to and detaching from their surface. This is mediated by lectin-binding glycoproteins, L-selectin on the surface of neutrophils and E-selectins on the endothelial cell.

In relation to infections, the neutrophils respond to chemoattractants such as complement components, IL-8, platelet activating factor and microbial peptides generated at the infection site leading to activation of integrins present on the surface of neutrophils. These integrins subsequently induce tighter adherence of neutrophils to vascular endothelial cells and cessation of rolling. Neutrophils then detach from endothelium and migrate through a gap in the endothelial lining created by contraction of juxtaposed vascular endothelial cells. After their migration into the extravascular space, neutrophils proceed with the ingestion and killing of microbes. This proceeds in three steps, namely adhesion, opsonization and ingestion and intracellular killing.

The various components of neutrophil functions (Wilkinson, 1981; Metcalf *et al.,* 1986; Ryder, 2010; Kunhs, 2008, Dale *et al.*, 2008) are tested in the laboratory by using several and simple economical tests which yield valuable information regarding neutrophil functional abnormalities in periodontitis.

Isolation of the neutrophils

For laboratory assay of neutrophils, a relatively pure population of granulocytes or at least all white blood cells with minimum manipulation of blood are preferred. The blood sample is collected either with heparin or EDTA as anticoagulant. Most of the isolation protocols use differences in cell density as the basis for separation. A 3 % solution of

gelatin or high molecular weight dextran in saline is the most preferred method because it gives a relatively pure population of WBCs with the minimal contamination with the RBCs. To obtain granulocytes, Ficoll-Hypaque is most commonly used.

For the separation of WBCs, the blood sample is diluted with saline and equal amount of 3 % gelatin or dextran is added. The tubes are made to stand upright for 45 min till all the RBCs settle down. The supernatant is collected in a fresh tube, centrifuged and washed with saline, and the WBCs are counted in Neubauer's chamber and then diluted with Hanks balanced salt solution to get a specific concentration depending on the type of test to be performed.

Neutrophil adherence

Adherence of neutrophils to the endothelium is a prerequisite step to a migration of neutrophils into the tissues. The adhesive function is commonly assessed by the passage of 1 mL of the whole blood through the column filled with nylon wool. Adherence is measured as the difference in the absolute neutrophil count before and after the passage of the sample through the column. Alternatively, isolated neutrophils are induced as to bind to plastic using a 96-well plate coated with foetal bovine serum, fibrinogen or fibronectin. The adherent activity of the cells can be promoted by treatment with phorbol myristate acetate, and the results can be analysed after staining.

Neutrophil chemotaxis

Neutrophil migration is a prerequisite for these cells to accumulate at sites of inflammation. Chemotaxis in vitro is generally measured by one of the two methods—Boyden's chamber technique or agarose assay. The Boyden's chamber includes three components: a lower compartment containing chemoattractant agent, middle nitrocellulose membrane filter layer and an upper chamber for neutrophils. After adding cell suspension to the upper chamber and incubation for a specified period of time, the membrane filter is removed, stained and studied under the microscope to find out the number of cells that have adhered to the membrane and trying to pass through the narrow pores.

The agarose assay is popular, because it is simpler to perform and the results are comparable to those of Boyden's assay. Here, 0.24 % agarose is prepared, melted and mixed with pooled human serum. Hanks balanced salt solution and sodium bicarbonate solution poured onto microscopic slides when still hot. Once the slides were set, a series of wells of 3–5 mm were cut in the agarose layer. The central well was charged with cells, and the peripheral wells were loaded with Hanks solution on one side and FMLP a known chemotactic agent on the other. The slides are incubated at 37 °C for 2 h and fixed first with methanol and then with formaldehyde. The agarose layer was scraped off, and the slide was stained with Giemsa stain. The movements of the cells were observed as a tongue-shaped structure with the narrow end away from the central well and towards the peripheral well containing FMLP. The distance migrated was measured under the microscope using oculometer.

Generation of reactive oxygen intermediate (NBT assay test)

The release of reactive oxygen intermediates (ROIs) such as superoxide radicals and hydrogen peroxide is an important component of neutrophil's bactericidal machinery. These reactions are mediated by NADPH oxidase, and the neutrophils defective in this enzyme cannot generate ROI leading to defective microbial killing.

The production of superoxide can be tested by reduction of cytochrome C, and the extracellular release of H_2O_2 can be measured by horse radish peroxidase-induced oxidation of phenol red. The most common test used for this purpose is nitro blue tetrazolium (NBT) assay. The cells are exposed to the yellow dye NBT. Unstimulated neutrophils do not ingest this dye, but if the cells are stimulated to phagocytic activity, then they take the dye into the phagosomes and intracellular reduction of the dye and convert it to an insoluble blue crystalline form (formazan crystals). These blue crystals are visible in the light microscope. This test gives information about phagocytic function since the dye is not taken into cells except by phagocytosis and also about metabolic function since the intracellular reduction depends upon the production of ROIs.

In NBT test, two aliquots of whole blood were mixed separately with Hanks' salt solution and 0.34 % NBT dye. *Escherichia coli endotoxin* was added to one aliquot to stimulate the neutrophils, and the other was left unstimulated to act as a control. After incubation for about 45 min, smears are prepared from both test and control samples and stained with Giemsa and the number of cells with formazan crystals was noted. [In a normal sample, only few cells in control (unstimulated) and >60 % cells in test (stimulated) should show the presence of crystals.]

The method employed nitro blue tetrazolium (NBT) assay to analyse the reactive oxygen intermediates, hence the name NBT assay. *Escherichia coli endotoxin*/test compound was the positive control (PC), and the test compound without *E. coli endotoxin* was the negative control (NC).

Phagocytosis

Most methods for measuring phagocytosis rely on the uptake of particles by phagocytes over a brief period of time. The number of particles are then counted under the microscope and expressed as mean particle number (MPN).

In this assay, neutrophil suspension was mixed with equal portions of cooled normal human serum, Hanks' solution and *Candida albicans* or latex particles and incubated at 37 °C for 30 min. The tubes were then centrifuged; smears were made from the deposit. Most methods for measuring phagocytosis relied on the uptake of particles by phagocytes over a brief period of time. The number of particles was then counted under the microscope and expressed as mean particle number (MPN).

Microbicidal (candidacial) assay

This test is performed to assess the microbicidal or intracellular killing capacity of neutrophils. Here, the WBC suspension was mixed with Hanks' solution, pooled serum and *Candida cells* as mentioned above, and the test and control samples were incubated for an hour at 37 °C. Equal portion of 2.5 % sodium deoxycholate was then added and mixed properly. This leads to lyses of neutrophil membranes and releases ingested *candida* to the exterior. The samples were then mixed with 0.01 % methylene blue solution; centrifuged and wet preparations were made from the deposit and examined under the microscope. Viable *candida* remained unstained where as dead cells took up the dye and appeared blue (In a healthy person, the normal range of killing should be around 20–35 %). The cooled human serum/test compound was the positive control (PC), and the negative control (NC) was test compound without serum.

Results and discussion

Chemistry

Thiocarbohydrazide (**1**) and acetic acid (**2**) were refluxed for 8 h (Scheme 1) to yield 4-amino-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (**3**). The FT-IR spectra in KBr pellets confirmed the asymmetric and symmetric stretches for $-NH_2$ at 3342 and, 3270 cm^{-1}. The –C–H stretch of $-CH_3$ was at 2942 cm^{-1} and –SH stretch at 2580 cm^{-1}. The 400 MHz ^{1}H-NMR and ^{13}C-NMR recorded in DMSO-d_6, δ ppm downfield from TMS confirmed the formation of 4-amino-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (**3**) which was further confirmed by LC-Mass spectra, where the compound signalled protonated molecular ion peak, i.e. $[M^+ + 1]$ peak at (m/z): 131.00.

4-Amino-3-methyl-5-mercapto-4*H*-[1,2,4]triazole (**3**) was condensed with different aromatic aldehydes (**4**) to yield the resultant series of novel Schiff bases, namely 4-[(benzylidene)amino]-3-methyl-5-mercapto-4*H*-[1,2,4]-triazole (**5a–l**) (Scheme 1). The FT-IR spectra in KBr pellets gave peaks in the region 3040–3080, 2920–2960 and 2650–2760 cm^{-1} corresponding to >C–H stretch of aryl group, –C–H stretch of $-CH_3$ group and –SH stretch, respectively. In the novel Schiff bases (**5a–l**), the absence of peaks for $-NH_2$ of the compound (**3**) was the indication for the formation of Schiff bases. In addition to that, the formation of benzylidene moiety (–N=CH–Ar) was confirmed which was further supported by 400 MHz ^{1}H-NMR and ^{13}C-NMR recorded in DMSO-d_6, δ ppm downfield from TMS. The benzylidene proton appeared in the region δ 10.50–10.90 ppm. The benzylidene carbon resonated at δ 160.00–165.00 ppm. The LC-Mass spectra were in conformity with the corresponding molecular formulae of the novel compounds. The structures of the novel compounds (**5a–l**) were confirmed by spectral and elemental analyses.

Pharmacological screening

Antituberculosis activity

The Schiff bases (**5a–l**) possess antituberculosis activity for in vitro test system but with different sensitivity (Tables 2, 3). The antituberculosis activity by MABA method (MIC test) revealed that all the compounds of the novel series exhibited excellent sensitivity up to 50 μg/mL, and in the ZOI tests, the tested compounds exhibit moderate activity almost half to the reference standards. The compounds (**5a**), (**5c**), (**5f**), (**5h**) and (**5i**) are most sensitive in the series, but are moderately active compared to the reference standards. These compounds were subjected to the acute oral toxicity studies, where they belong to the class of slightly toxic. The compounds should be tested further to consider them as potential antituberculosis drug candidate. The presence of halogens on phenyl ring may be the additional factor for the excellent activity of the compounds. The structure activity relationships (SAR) of numerous FDA-approved drugs confirmed that the presence of electronegative halogen atoms (especially fluorine or chlorine) augments or contributes to the biological activity. It is proved by number of researchers that halogen compounds exhibit excellent pharmacological results due to its electronegativity factor. The reference standards **pyrazinamide** (specific antituberculosis drug), **streptomycin** and **ciprofloxacin** (antibiotics and broad-spectral antituberculosis drugs) were highly sensitive against *M. tuberculosis* (H37RV) strain. However, tested compounds cannot directly be compared with the reference standards for their efficacy since it depends on therapeutic index,

Table 2 Antituberculosis activity of Schiff bases (**5a–l**) by disc diffusion method (ZOI test)

Samples	Diameter of inhibition zone (mm ± SD)[a] *Mycobacterium tuberculosis* (H37RV) strain
5a	13.00 ± 0.25
5b	9.00 ± 0.35
5c	13.50 ± 0.25
5d	11.25 ± 0.35
5e	8.50 ± 0.30
5f	13.50 ± 0.40
5g	12.60 ± 0.20
5h	13.00 ± 0.35
5i	13.50 ± 0.40
5j	12.00 ± 0.35
5k	11.50 ± 0.30
5l	12.00 ± 0.45

'0' indicating no sensitivity (zone of inhibition <5 mm)

Reference standards: pyrazinamide: 30.00 ± 0.65 mm, ciprofloxacin: 27.00 ± 0.45 mm, streptomycin: 27.00 ± 0.55 mm

[a] Mean values of three trials

which varies for different bioactive compounds depending upon the ratio between lethal dose and therapeutic dose.

Neutrophil function test

The novel Schiff bases (**5a–l**) were subjected to the various components of neutrophil function, namely neutrophil chemotaxis, NBT test, phagocytosis and microbicidal (candidacial) assay test according to the simple and well-established procedure. The tested novel compounds possess excellent activity with different sensitivity under different segments. Tabulating the results of the different components under the collective title neutrophil function test, the novel Schiff bases (**5a–l**) exhibited moderate to excellent activities. Considering the individual components, i.e. chemotaxis (>1.0), NBT (>25 %), phagocytosis (>3) and candidacial assay (25 %) possessing excellent broad-spectrum properties, the compounds (**5a**), (**5b**), (**5g**) and (**5h**) exhibited excellent neutrophil function test and can be considered potential drug candidates after being subjected to further tests. The data are given in Table 4.

Toxicity studies

The novel Schiff bases, (**5a**), (**5c**), (**5f**), (**5h**) and (**5i**) which exhibited moderate antituberculosis activity and (**5a**), (**5b**), (**5g**) and (**5h**) which showed excellent neutrophil function test, were analysed for acute oral toxicity considering their future potential utilities and safer mode of exposures. The acute oral toxicity was done according to the OECD/OCDE 423 guidelines (retrieved on 10.08.2014). Handling and animal experimentation were done according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA, 2001), India (EPA 815-R-08-003, retrieved on 18.02.2014), with the ethical clearance certificate KLECOP/IAEC/Res. 18–19/05/2014. The acute toxicity was tested as per the OECD 423 guidelines for the period of 14 days on Swiss albino mice (*Mus musculus*) in administering the doses of 5, 50, 300 and 2000 mg/kg body weight where the test animals showed the normal behavioural pattern without developing any complications. Finally from the acute

Table 3 Antituberculosis activity of Schiff bases (**5a–l**) by microplate Alamar Blue assay method (MABA) (MIC test)

Samples	100 μg/mL	50 μg/mL	25 μg/mL	12.5 μg/mL	6.25 μg/mL	3.12 μg/mL
5a	**S**	**S**	R	R	R	R
5b	**S**	**S**	R	R	R	R
5c	**S**	**S**	R	R	R	R
5d	**S**	**S**	R	R	R	R
5e	**S**	**S**	R	R	R	R
5f	**S**	**S**	R	R	R	R
5g	**S**	**S**	R	R	R	R
5h	**S**	**S**	R	R	R	R
5i	**S**	**S**	R	R	R	R
5j	**S**	**S**	R	R	R	R
5k	**S**	**S**	R	R	R	R
5l	**S**	**S**	R	R	R	R

Bacterial strain: *M. tuberculosis* (H37RV) strain

Reference standards: pyrazinamide: 3.125 μg/mL, ciprofloxacin: 3.125 μg/mL, streptomycin: 6.25 μg/mL

S sensitive, *R* resistant

Table 4 Neutrophil function test of Schiff bases (**5a–l**)

Samples	Neutrophil chemotaxis (mm)	NBT (%)	Phagocytosis (MPN)	Microbicidal (candidacial) assay (%)
5a	2.2	30	3	32
5b	2.1	30	4	30
5c	2.4	19	3.5	30
5d	2.6	21	3.5	34
5e	2.3	20	3	32
5f	2.2	22	2.5	31
5g	2.4	30	3	30
5h	2.7	32	4	37
5i	2.3	18	3	30
5j	2.4	18	3	31
5k	2.3	15	3.5	28
5l	2.3	19	2.5	30
NC	0.5	20	3	16
PC	2.2	65	5	30

For neutrophil chemotaxis: PC—test compound/FMLP, NC—WBC suspended in the media. For NBT: PC—*E. coli endotoxin*/test compound, NC—test compound without *E. coli endotoxin*. For phagocytosis: PC—cooled normal human serum/test compound, NC—test compound without serum. For candidacial assay: PC—serum/test compound, NC—test compound without serum

Control: *NC* negative control, *PC* positive control

toxicity studies, it is revealed that the compounds (**5a**), (**5b**), (**5c**), (**5f**), (**5g**), (**5h**) and (**5i**) were safe even at the dose of 2000 mg/kg body weight. Hence, as per the Hodge and Sterner scale toxicity (CPCSEA, 2001), these compounds can be classified as slightly toxic as far as mammalian oral route of exposure is concerned. The acute toxicity study values obtained are of use for determination of therapeutic index in view of their relatively good antituberculosis activity and neutrophil function test.

Conclusion

The present study reveals that the newly synthesized Schiff bases of [1,2,4]triazole-bearing haloarene moiety (**5a–l**) possess moderate biological activity. The results convey the successful synthesis and good activity of novel compounds. In the series, the compounds (**5a**) and (**5h**) have two fluorine atoms at positions 2 and 4 that exhibit the highest activity for antituberculosis screening as well as for neutrophil function test. In addition, all the novel Schiff bases serve as intermediates for Schiff and Mannich bases and may contribute to the development of potential antituberculosis drug. The compounds (**5a**), (**5b**), (**5c**), (**5f**), (**5g**), (**5h**) and (**5i**) which exhibited moderate activities subjected to acute oral toxicity studies revealed that they are 'slightly toxic' which stimulates for further studies for the potential use of these lead compounds.

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