5.1 Introduction

This chapter covers preparation of novel analogues of fluorine containing pyrazole based 5-(phenylthio)substituted-triazolo[4,3-a]pyrimidine derivatives under conventional method and their biological evaluation. The titled compounds were prepared by the reacting appropriate chalcone and 5-substituted-1H-1,2,4-triazol-3-amine in basic media with DMF (dimethyl formamide) as solvent. The corresponding chalcone derivatives were prepared by reacting 3-methyl-5-substitutedphenylthio-1-phenyl-1H-pyrazole-4-carbaldehydes and fluoro acetophenones. $^1$H NMR, $^{13}$C NMR, IR, mass spectrometric techniques have been employed to characterize synthesized compounds. In vitro antioxidant, antibacterial, antimalarial and antitubercular activities of these compounds were evaluated. Many members of this pyrazolo pyrimidine class possessed noteworthy activities comparable to the standards.

5.2 Literature survey on 5-(phenylthio)-substituted-triazolo[4,3-a]pyrimidine derivatives

A. Kumar and his teammates[1] reported the preparation of 5,7-Diarylpyrazolo[1,5-a]pyrimidines using KOH mediated tandem type reaction between variously substituted chalcone and 1H-pyrazol-3-amines. Authors had screened many base-solvent pair, but out of them KOH in DMF was reported to be the most prominent for the high yield in shorter time period (Scheme 5.1).

M. Khera et al.[2] synthesized novel oxazolidinone motifs having 1,2,4-triazolo[4,3-a]pyrimidine core moiety. These derivatives were screened for their antimicrobial
activity. Authors had compared these synthesized compounds with other compounds of oxazolidinone class such like Linezolid and Eperezolid for their activity (Figure 5.1).

Figure 5.1 Biologically active novel oxazolidinone derivatives based on 1,2,4-triazolo[4,3-a]pyrimidine

J. Bhatt and his team[3] reported the synthesis of pyrazole based triazolo[1,5-a]pyrimidine hybrids. Their antituberculosis potency was screened. Authors had also performed molecular docking for the synthesized compounds. Some of the compounds got bound completely to the pocket (active site) of receptor and showed fine docking score (Scheme 5.2).

Scheme 5.2 Synthesis of triazolopyrimidines as an antitubercular agent

A. Sharma and his team[4] synthesized pyrimidyl hydrazones and 3-(quinolin-3-yl)-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidine compounds under routine condition. Authors had reported molecular docking score and DNA-photo cleavage activity of the compounds (Scheme 5.3).
S. Sirakanyan et al. [5] synthesized novel library of pyrido[3′,2′:4,5]furo[3,2-d][1,2,4]triazolo[4,3-a]pyrimidin-7(8)-one derivatives. Spectroscopic methods were employed for the structural characterization (Scheme 5.4).

H. Liu et al. [6] synthesized [1,2,3]-triazolo[4,5-d]pyrimidine/thiourea hybrids to check their preliminary antimicrobial potency. Some compounds of this prepared library exhibited good inhibition of the cell growth of the lungs cancerous cell and hence established to be good antiproliferative agents (Scheme 5.5).
M. Bakavoli and his team[7] reported preparation of oxazolo[5,4-
\(d\)][1,2,4]triazolo[4,3-\(a\)]pyrimidines by conventional method (Scheme 5.6).

X. Liu et al.[8] developed easy preparation of 1,2,4-triazolo[4,3-\(a\)]pyridin-3(2\(H\))-one under microwave method to check their herbicidal activity (Scheme 5.7).

R = 4-CN PhCH\(_2\), 4-Cl PhCH\(_2\), 4-OMe PhCH\(_2\), 3-Cl PhCH\(_2\), -PhCH\(_2\), 2-Cl PhCH\(_2\), 2-F PhCH\(_2\), 4-t-Bu PhCH\(_2\), -CH\(_2\)CN, -Propyl, -Undecyl, -Butyl, -Allyl

**Scheme 5.5** Synthesis of antiproliferative active [1-3]triazolo [4,5-\(d\)]pyrimidine/thiourea hybrids

**Scheme 5.6** Synthesis of oxazolo[5,4-\(d\)][1,2,4]triazolo[4,3-\(a\)]pyrimidines derivatives

**Scheme 5.7** Synthesis of herbicidally active 1,2,4-triazolo[4,3-\(a\)]pyridin-3(2\(H\))-one derivatives
S. Abou-Seri and his co-workers\cite{9} reported preparation of pyrido[2,3-\textit{d}]pyrimidine and pyrido[2,3-\textit{d}][1,2,4]triazolo[4,3-\textit{a}]pyrimidine derivatives. The authors had characterized these compounds. They also screened these compounds for antitumor and apoptosis activity through G1 cell-cycle (Scheme 5.8).

\textit{Ar} = -C_{6}H_{5}, 4-FC_{6}H_{4}, 4-ClC_{6}H_{4}, 4-MeC_{6}H_{4}, 4-OMeC_{6}H_{4}

\textbf{Scheme 5.8} Synthesis and antitumor activity of pyrido[2,3-\textit{d}]pyrimidine and pyrido[2,3-\textit{d}][1,2,4]triazolo[4,3-\textit{a}]pyrimidine derivatives

G. Alzué-Piña \textit{et al.}\cite{10} synthesized triazolopyridopyrimidine compounds and checked their antileishmanial and DNA binding activity. These compounds were very effective DNA photocleavers (Scheme 5.9).

\textbf{Scheme 5.9} Synthesis of triazolopyrimidines

T. Parish and her co-workers\cite{11} prepared di-substituted triazolopyrimidine and checked their antituberculosis activity. They also studied structure activity relationship, physicochemical parameters and cytotoxic activity against eukaryotic cells of these compounds (Scheme 5.10).
A. Kamal and his team[12] reported pyrazolo[1,5-α]pyrimidines compounds having chalcone linkage in its structure. Molecular docking was performed for the synthesized derivatives and they were screened for their anticancer potency (Scheme 5.11).

They have characterized the compounds using spectroscopic techniques. They also evaluated antitumor activity of the synthesized derivatives (Scheme 5.12).

**5.3 Present work**

Pyrimidine ring fused with triazole moiety commonly known as triazolopyrimidine is the fused aza-heterocycle having large pharmacological activity. It has four nitrogen atoms present in the ring structure. The multicomponent preparation of triazolopyrimidine scaffolds has been reported. They exhibited wide variety of medicinal purpose including allosteric modulator[14], anticonvulsant[15], antibacterial[2, 16, 17], anti-alzheimer’s[18], antitumor[3, 11-13], herbicidal[8], antileishmanial activity[10], antifungal[19], antimicrobial[20], antiproliferative[6], apoptosis inducer[9] and DNA-photoclavager[21]. Apart from these, pyrazolopyrimidine compounds moreover showed various medicinal applications like anticancer[12, 22], anti-inflammatory[23], antimicrobial[23], antimalarial[24], dehydrogenase inhibitors[24], antitumor[9, 25] and antimalarial[24] activities.

Fluorine substituted heterocycles plays very important role[26-28] in enhancing pharmacokinetic and pharmacodynamic properties of drugs molecules. The most significant approach to enhance biological and physical properties is due to introduction of the trifluoromethylation group. This increases *in vitro* uptake and hence transport the drug molecule[29, 30] due to its high lipophilicity. Fluoro substituted pyrazole combined with triazolopyrimidine moiety displayed many medicinal applications as discussed above.
In this perception, the work described in present thesis composed of designing and preparation of pyrazole combined with fluoro-substituted triazolopyrimidine moiety by reacting \(\alpha,\beta\)-unsaturated ketone and 5-substituted-1\(H\)-1,2,4-triazol-3-amine in presence of KOH (potassium hydroxide) in DMF under conventional method.

**5.4 Reaction scheme**

The preparation of titled derivatives is described in **Scheme 5.13**. 5-chloro-3-methyl-1-phenyl-1\(H\)-pyrazole-4-carbaldehyde 1 was prepared as per the method reported earlier[31]. The resultant 5-((4-substituted-phenyl)thio)-3-methyl-1-phenyl-1\(H\)-pyrazole-4-carbaldehydes 3a-e were formed by refluxing compound 1 and thiophenols 2a-e using anhydrous K\(_2\)CO\(_3\) as basic catalyst in DMF. Then the resultant aldehydes 3a-e were allowed to react with the fluorosubstituted acetophenone 3a'-b' in ethanolic NaOH to obtain chalcone derivatives i.e. 4a-j. Differently substituted chalcones 4a-j were then reacted with 5-substituted-1\(H\)-1,2,4-triazol-3-amine 5a-b in the presence of KOH and DMF as solvent to yield the targeted compounds i.e. 6a-t.
### Substitutional variations of titled compounds (6a-t)

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<th>Yield&lt;sup&gt;a&lt;/sup&gt;(%)</th>
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<sup>a</sup> Isolated yield

### 5.5 Experimental procedure

#### 5.5.1 Synthesis of 5-(phenylthio) substituted triazolo [4,3-a]pyrimidine derivatives (6a-t)

The targeted compounds (6a-t) were prepared using following steps:

##### 5.5.1.1 Synthesis of 3-methyl-5-substituted phenylthio-1-phenyl-1H-pyrazole-4-carbaldehydes (3a-e)

The synthetic procedure of carbaldehyde 3a-e is already described in chapter 2 (Section-2.6.1.1).

##### 5.5.1.2 Synthesis of (E)-3-(3-methyl-1-phenyl-5-(p-substitutedphenylthio)-1H-pyrazol-4-yl)-1-(p-substitutedphenyl)prop-2-en-1-one (4a-j)
Thiosubstituted-aldehyde $3\text{a-e}$ (1 mmol) was stirred in 20 ml ethanolic solution at ambient temperature in round bottom flask to homogeneous mixture. After that substituted-acetaldehyde $3\text{a'-b'}$ (1 mmol) was slowly added to this homogeneous solution keeping the flask in ice-bath. Then after 20% NaOH solution was dropwise added to the mixture. After complete reaction, as noticed by TLC, ice bath was removed. The mixture was stirred for 30-35 min at normal temperature. The product was separated in form of precipitates. Then it was filtered and washed with cold ethanol. Crystallization was performed using hot ethanol to obtain pure $4\text{a-j}$.

5.5.1.3 Synthesis of 3-methyl-5-(3-methyl-1-phenyl-5-(p-substitutedphenylthio)-1H-pyrazol-4-yl)-7-(p-substitutedphenyl)-[1,2,4]-triazolo[4,3-a]pyrimidine (6a-t)

α,β-unsaturated ketone $4\text{a-j}$ (1 mmol) and 5-substituted-1H-1,2,4-triazol-3-amine $5\text{a-b}$ (1 mmol) were mixed in DMF. To this solution, 10% KOH (2 mmol) solution was added and refluxed for 1.5-2 hour. After complete reaction, as noticed by TLC, reaction mixture was cooled and poured into cold water. The product was precipitated. It was then filtered and washed with cold water. Crystallization was performed from hot ethanol to afford $6\text{a-t}$ in good yield.

5.6 Preliminary and Spectral Characterization

Various spectroscopic methods have been employed for structural confirmation of compounds $6\text{a-t}$. The characteristic C–H stretching of aromatic ring was observed at around 3074-2891 cm$^{-1}$. The IR spectrum of $6\text{a-t}$ showed absorption in the range of 1275-1126 cm$^{-1}$ due to aromatic C=N stretching. The confirmation of various protons and carbons was achieved by proton NMR and carbon NMR spectroscopy. The chemical structures are established by molecular ion (Base peak) peak corresponding to respective molecular weights in mass spectra.

The spectral data for structural characterization of prepared compounds $6\text{a-t}$ are described in tables spread over following few pages.
7-(4-fluorophenyl)-5-(3-methyl-1-phenyl-5-(phenylthio)-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula C_{27}H_{19}FN_{6}S
Molecular Weight (gm.mol⁻¹) 478.5
Melting Point (°C) 183-185

FT-IR ν_{max} cm⁻¹ (KBr): 3060, 1598, 1553, 1494, 1349, 1291, 1220, 1158, 995, 851, 695

^1H NMR (δ ppm, DMSO-d₆): 2.36 (s, 3H, -CH₃ of pyrazole), 6.88 (t, 2H, Ar-H), 7.66 (s, 3H, Ar-H), 7.43 (m, 2H, Ar-H), 7.53 (m, 5H,Ar-H), 7.94 (s, 1H, Ar-H), 8.23 (m, 2H, Ar-H), 8.71 (s, 1H, triazole-H)

^13C NMR (δ ppm, DMSO-d₆): 13.3, 109.2, 116.0, 116.2, 116.6, 125.3, 127.0, 127.92, 128.76, 129.0, 129.1, 130.0, 130.1, 132.3, 132.4, 133.1, 133.6, 138.3, 140.7, 149.3, 155.0, 155.9, 158.8, 162.9, 165.3

Elemental Analysis  Actual: C, 67.77; H, 4.00; N, 17.56%
Found: C, 67.78; H, 4.01; N, 17.54%

7-(2,4-difluorophenyl)-5-(3-methyl-1-phenyl-5-(phenylthio)-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula C_{27}H_{19}F_{2}N_{6}S
Molecular Weight (gm.mol⁻¹) 496.5
Melting Point (°C) 195-196

FT-IR ν_{max} cm⁻¹ (KBr): 3062, 1595, 1554, 1496, 1357, 1290, 1224, 1160, 994, 861, 778, 687

^1H NMR (δ ppm, DMSO-d₆): 2.36 (s, 3H, -CH₃ of pyrazole), 6.89 (t, 2H, Ar-H), 7.07 (s, 3H, Ar-H), 7.43 (m, 2H, Ar-H), 7.54 (m, 4H,Ar-H), 7.94 (s, 1H, Ar-H), 8.24 (m, 2H, Ar-H), 8.71 (s, 1H, triazole-H)

^13C NMR (δ ppm, DMSO-d₆): 13.3, 109.3, 116.1, 116.3, 116.5, 125.4, 127.1, 127.8, 128.8, 129.1, 129.2, 130.1, 132.3, 132.4, 133.2, 133.5, 138.3, 140.8, 149.3, 155.1, 155.9, 158.8, 160.1, 162.8, 165.3

Elemental Analysis  Actual: C, 65.31; H, 3.65; N, 16.93%
Found: C, 65.32; H, 3.68; N, 16.95%
5-(phenylthio)substituted triazolo[4,3-a]pyrimidine

Molecular Formula: C_{28}H_{21}FN_6S
Molecular Weight (g.mol^{-1}): 492.5
Melting Point (°C): 187-189

FT-IR \ \upsilon_{\text{max}} \ \text{cm}^{-1} \ (\text{KBr}): 3056, 1588, 1555, 1490, 1367, 1286, 1221, 1155, 998, 852, 692

\textsuperscript{1}H NMR (δ ppm, DMSO-d_6): 2.35 (s, 3H, -CH_3 of pyrazole), 2.96 (s, 3H, triazole-CH_3), 6.78 (m, 5H, Ar-H), 7.48 (m, 6H, Ar-H), 7.89 (m, 2H, Ar-H), 8.05 (m, 2H, Ar-H)

\textsuperscript{13}C NMR (δ ppm, DMSO-d_6): 13.1, 17.6, 109.2, 115.8, 116.1, 116.2, 125.3, 125.6, 128.7, 128.9, 129.0, 129.1, 129.6, 130.1, 130.7, 132.4, 132.5, 134.7, 138.4, 140.6, 149.3, 155.1, 155.7, 162.9, 165.4

Elemental Analysis
- Actual: C, 68.28; H, 4.30; N, 17.06%
- Found: C, 68.30; H, 4.29; N, 17.05%

---

7-(2,4-difluorophenyl)-3-methyl-5-(3-methyl-1-phenyl-5-(phenylthio)
-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula: C_{28}H_{20}F_2N_6S
Molecular Weight (g.mol^{-1}): 510.5
Melting Point (°C): 201-203

FT-IR \ \upsilon_{\text{max}} \ \text{cm}^{-1} \ (\text{KBr}): 3063, 1597, 1560, 1495, 1363, 1288, 1225, 1164, 995, 867, 782, 686

\textsuperscript{1}H NMR (δ ppm, DMSO-d_6): 2.35 (s, 3H, -CH_3 of pyrazole), 2.94 (s, 3H, triazole-CH_3), 6.87 (t, 2H, Ar-H), 7.12 (m, 3H, Ar-H), 7.42 (m, 2H, Ar-H), 7.56 (m, 4H, Ar-H), 7.95 (s, 1H, Ar-H), 8.25 (m, 2H, Ar-H)

\textsuperscript{13}C NMR (δ ppm, DMSO-d_6): 13.3, 17.6, 116.2, 116.4, 116.5, 125.5, 127.2, 127.7, 128.9, 129.1, 129.3, 130.2, 132.3, 132.5, 133.1, 133.6, 138.2, 140.7, 149.4, 155.2, 155.6, 158.9, 160.2, 162.7, 165.4

Elemental Analysis
- Actual: C, 65.87; H, 3.95; N, 16.46%
- Found: C, 65.88; H, 3.94; N, 16.47%
5-(phenylthio)substituted triazolo[4,3-a]pyrimidine

7-(4-fluorophenyl)-5-(3-methyl-1-phenyl-5-(p-tolylthio)-1H-pyrazol-4-yl)-1,2,4-triazolo[4,3-a]pyrimidine

Molecular Formula: C_{28}H_{21}FN_{6}S
Molecular Weight (g.mol\(^{-1}\)): 492.5
Melting Point (°C): 180-182

FT-IR \( \nu_{\text{max}} \) cm\(^{-1} \) (KBr): 3053, 1593, 1557, 1492, 1372, 1287, 1217, 1158, 992, 854, 697

\(^1\)H NMR (δ ppm, DMSO-d\(_6\)): 2.03 (s, 3H, Ph-CH\(_3\)), 2.34 (s, 3H, -CH\(_3\) of pyrazole), 6.87 (t, 2H, Ar-H), 7.07 (m, 3H, Ar-H), 7.44 (m, 2H, Ar-H), 7.52 (m, 4H, Ar-H), 7.95 (s, 1H, Ar-H), 8.22 (m, 2H, Ar-H), 8.72 (s, 1H, triazole-H)

\(^13\)C NMR (δ ppm, DMSO-d\(_6\)): 13.2, 20.2, 109.3, 116.1, 116.3, 116.5, 125.4, 127.1, 127.8, 128.8, 129.0, 129.2, 130.1, 130.3, 132.4, 132.5, 133.2, 133.7, 138.4, 140.7, 149.3, 155.1, 155.8, 158.9, 162.8, 165.3

Elemental Analysis
Actual: C, 68.28; H, 4.30; N, 17.06%
Found: C, 68.29; H, 4.31; N, 17.08%

---

7-(2,4-difluorophenyl)-5-(3-methyl-1-phenyl-5-(p-tolylthio)-1H-pyrazol-4-yl)-1,2,4-triazolo[4,3-a]pyrimidine

Molecular Formula: C_{28}H_{20}F\(_2\)N\(_6\)S
Molecular Weight (g.mol\(^{-1}\)): 510.5
Melting Point (°C): 196-198

FT-IR \( \nu_{\text{max}} \) cm\(^{-1} \) (KBr): 3061, 1560, 1560, 1498, 1365, 1281, 1230, 1163, 991, 866, 783, 680

\(^1\)H NMR (δ ppm, DMSO-d\(_6\)): 2.04 (s, 3H, Ph-CH\(_3\)), 2.35 (s, 3H, -CH\(_3\) of pyrazole), 6.88 (t, 2H, Ar-H), 7.06 (m, 3H, Ar-H), 7.45 (m, 2H, Ar-H), 7.56 (m, 3H, Ar-H), 7.93 (s, 1H, Ar-H), 8.25 (m, 2H, Ar-H), 8.72 (s, 1H, triazole-H)


Elemental Analysis
Actual: C, 65.28; H, 3.95; N, 16.46%
Found: C, 65.27; H, 3.96; N, 16.49%
7-(4-fluorophenyl)-3-methyl-5-(3-methyl-1-phenyl-5-(p-tolylthio) -1H-pyrazol-4-yl)12,4]triazolo[4,3-a]pyrimidine

Molecular Formula C_{29}H_{23}FN_{6}S
Molecular Weight (gm.mol^{-1}) 506.6
Melting Point (°C) 190-192

FT-IR  υ_{max} Cm^{-1} (KBr): 3069, 1659, 1588, 1491, 1389, 1285, 1219, 1094, 991, 847, 695

^1H NMR (δ ppm, DMSO-d_{6}): 2.04 (s, 3H, Ph-CH_{3}), 2.34 (s, 3H, -CH_{3} of pyrazole), 2.94 (s, 3H, triazole-CH_{3}), 6.79 (m, 5H, Ar-H), 7.46 (m, 6H, Ar-H), 7.90 (s, 1H, Ar-H), 8.06 (m, 2H, Ar-H)

^13C NMR (δ ppm, DMSO-d_{6}): 13.1, 17.6, 20.2, 109.7, 116.1, 116.3, 125.4, 128.7, 129.8, 129.9, 129.3, 129.7, 130.2, 130.8, 132.3, 132.4, 134.8, 137.9, 138.3, 140.5, 149.4, 155.2, 155.8, 162.8, 165.3

Elemental Analysis  Actual: C, 68.76; H, 4.58; N, 16.59%
Found: C, 68.74; H, 4.57; N, 16.60%

7-(2,4-difluorophenyl)-3-methyl-5-(3-methyl-1-phenyl-5-(p-tolylthio)-1H-pyrazol-4-yl)-12,4]triazolo[4,3-a]pyrimidine

Molecular Formula C_{29}H_{23}F_{5}N_{6}S
Molecular Weight (gm.mol^{-1}) 524.5
Melting Point (°C) 213-215

FT-IR  υ_{max} Cm^{-1} (KBr): 3054, 1661, 1595, 1492, 1391, 1282, 1225, 1096, 989, 846, 758, 692

^1H NMR (δ ppm, DMSO-d_{6}): 2.04 (s, 3H, Ph-CH_{3}), 2.33 (s, 3H, -CH_{3} of pyrazole), 2.95 (s, 3H, triazole-CH_{3}), 6.77 (m, 4H, Ar-H), 7.49 (m, 7H, Ar-H), 7.87 (s, 1H, Ar-H), 8.07 (s, 1H, Ar-H)

^13C NMR (δ ppm, DMSO-d_{6}): 13.1, 17.5, 20.2, 109.3, 115.7, 116.0, 116.2, 125.3, 128.7, 128.8, 128.9, 129.0, 129.6, 130.0, 130.1, 132.41, 132.43, 134.7, 137.0, 138.3, 140.7, 149.2, 155.0, 155.8, 158.7, 162.8, 165.3

Elemental Analysis  Actual: C, 66.40; H, 4.23; N, 16.02%
Found: C, 66.42; H, 4.25; N, 16.03%
5-((4-chlorophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl) 
-7-([2,4-difluorophenyl]-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula: C<sub>27</sub>H<sub>16</sub>ClF<sub>2</sub>N<sub>4</sub>S
Molecular Weight (g.mol<sup>-1</sup>): 530.9
Melting Point (°C): 197-199

FT-IR $\nu_{\text{max}}$ cm<sup>-1</sup> (KBr): 3053, 1665, 1596, 1491, 1397, 1285, 1229, 1084, 981, 837, 751, 689

$^1$H NMR (δ ppm, CDCl<sub>3</sub>): 2.34 (s, 3H, -CH<sub>3</sub> of pyrazole), 6.88 (t, 2H, Ar-H), 7.05 (s, 3H, Ar-H), 7.46 (m, 2H, Ar-H), 7.55 (m, 2H, Ar-H), 7.94 (s, 2H, Ar-H), 8.24 (m, 2H, Ar-H), 8.73 (s, 1H, triazole-H)

$^{13}$C NMR (δ ppm, CDCl<sub>3</sub>): 13.1, 116.3, 116.5, 116.7, 125.4, 127.3, 127.8, 128.9, 129.3, 130.3, 131.3, 132.4, 132.7, 133.5, 138.6, 140.8, 149.5, 155.3, 155.9, 158.8, 160.2, 162.8, 165.3

Elemental Analysis
Actual: C, 61.07; H, 3.54; N, 16.38%
Found: C, 61.08; H, 3.55; N, 16.39%
5-(5-((4-chlorophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl) -7-(4-fluorophenyl)-3-methyl-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula $\text{C}_{20}\text{H}_{15}\text{ClF}_{2}\text{N}_{2}\text{S}$
Molecular Weight (gm.mol$^{-1}$) 545.0
Melting Point (°C) 195-197

FT-IR $v_{\text{max}}$ cm$^{-1}$ (KBr): 3057, 1660, 1590, 1495, 1389, 1289, 1227, 1087, 982, 840, 752, 687

$^1$H NMR (δ ppm, DMSO-$d_6$): 2.35 (s, 3H, -CH$_3$ of pyrazole), 2.95 (s, 3H, triazole-CH$_3$), 6.78 (m, 4H, Ar-H), 7.50 (m, 7H, Ar-H), 7.86 (s, 1H, Ar-H), 8.06 (t, 1H, Ar-H)

$^{13}$C NMR (δ ppm, DMSO-$d_6$): 13.2, 17.5, 109.2, 115.8, 116.1, 116.3, 125.4, 128.8, 128.9, 129.0, 129.2, 129.7, 130.2, 130.3, 132.3, 132.5, 134.8, 137.1, 138.2, 140.8, 149.3, 155.1, 155.9, 158.8, 159.7, 162.9, 165.3

Elemental Analysis Actual: C, 61.71; H, 3.51; N, 15.42%
Found: C, 61.73; H, 3.54; N, 15.45%

5-(5-((4-chlorophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl) -7-(4,5-difluorophenyl)-3-methyl-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula $\text{C}_{20}\text{H}_{15}\text{ClF}_{2}\text{N}_{2}\text{S}$
Molecular Weight (gm.mol$^{-1}$) 545.0
Melting Point (°C) 195-197

FT-IR $v_{\text{max}}$ cm$^{-1}$ (KBr): 3057, 1660, 1590, 1495, 1389, 1289, 1227, 1087, 982, 840, 752, 687

$^1$H NMR (δ ppm, DMSO-$d_6$): 2.35 (s, 3H, -CH$_3$ of pyrazole), 2.95 (s, 3H, triazole-CH$_3$), 6.78 (m, 4H, Ar-H), 7.50 (m, 7H, Ar-H), 7.86 (s, 1H, Ar-H), 8.06 (t, 1H, Ar-H)

$^{13}$C NMR (δ ppm, DMSO-$d_6$): 13.2, 17.5, 109.2, 115.8, 116.1, 116.3, 125.4, 128.8, 128.9, 129.0, 129.2, 129.7, 130.2, 130.3, 132.3, 132.5, 134.8, 137.1, 138.2, 140.8, 149.3, 155.1, 155.9, 158.8, 159.7, 162.9, 165.3

Elemental Analysis Actual: C, 61.71; H, 3.51; N, 15.42%
Found: C, 61.73; H, 3.54; N, 15.45%
7-(4-fluorophenyl)-5-(5-((4-fluorophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula C_{27}H_{16}F_{2}N_{6}S
Molecular Weight (g.m.mol⁻¹) 496.5
Melting Point (°C) 200-202

FT-IR \( \nu_{\text{max}} \text{ cm}^{-1} \) (KBr): 2981, 1620, 1588, 1552, 1488, 1367, 1293, 1219, 1072, 994, 778, 652

\(^1\text{H NMR}\) (δ ppm, CDCl₃): 2.40 (s, 3H, -CH₃ of pyrazole), 6.75 (d, 2H, Ar-H), 6.84 (d, 2H, Ar-H), 7.24 (m, 2H, Ar-H), 7.45 (m, 6H, Ar-H), 8.10 (m, 2H, Ar-H), 8.50 (s, 1H, triazole-H)

\(^{13}\text{C NMR}\) (δ ppm, DMSO): 13.1, 109.3, 116.9, 116.1, 125.4, 127.93, 128.77, 129.0, 130.1, 130.3, 131.3, 132.3, 132.4, 133.5, 133.8, 140.7, 149.2, 155.0, 155.9, 158.9, 159.9, 162.8, 165.4

Elemental Analysis
Actual: C, 65.31%; H, 3.65%; N, 16.93%
Found: C, 65.32%; H, 3.66%; N, 16.94%

7-(2,4-difluorophenyl)-5-(5-((4-fluorophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula C_{27}H_{14}F_{3}N_{6}S
Molecular Weight (g.m.mol⁻¹) 514.5
Melting Point (°C) 202-204

FT-IR \( \nu_{\text{max}} \text{ cm}^{-1} \) (KBr): 2987, 1630, 1594, 1558, 1481, 1371, 1283, 1224, 1069, 996, 782, 650

\(^1\text{H NMR}\) (δ ppm, DMSO-d₆): 2.35 (s, 3H, -CH₃ of pyrazole), 6.78 (t, 2H, Ar-H), 6.98 (s, 3H, Ar-H), 7.39 (m, 2H, Ar-H), 7.46 (m, 2H, Ar-H), 7.87 (s, 2H, Ar-H), 8.09 (m, 2H, Ar-H), 8.72 (s, 1H, triazole-H)

\(^{13}\text{C NMR}\) (δ ppm, DMSO-d₆): 13.1, 116.2, 116.6, 116.8, 125.3, 127.4, 127.8, 128.8, 129.2, 129.7, 130.2, 132.3, 132.8, 133.7, 138.5, 140.7, 149.4, 155.2, 155.8, 158.7, 159.4, 160.3, 162.9, 165.3

Elemental Analysis
Actual: C, 63.03%; H, 3.33%; N, 16.33%
Found: C, 63.05%; H, 3.32%; N, 16.32%
7-(4-fluorophenyl)-5-(5-((4-fluorophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-methyl-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula: C_{28}H_{20}F_{2}N_{6}S
Molecular Weight (g.mol\(^{-1}\)): 510.5
Melting Point (°C): 207-209

FT-IR \( \nu_{\text{max}} \) cm\(^{-1}\) (KBr): 2982, 1625, 1591, 1560, 1492, 1374, 1290, 1223, 1069, 998, 781, 654

\(^1\)H NMR (δ ppm, CDCl\(_3\)): 2.35 (s, 3H, -CH\(_3\) of pyrazole), 2.94 (s, 3H, triazole-CH\(_3\)), 6.78 (m, 5H, Ar-H), 7.48 (m, 4H, Ar-H), 7.84 (m, 3H, Ar-H), 8.06 (m, 2H, Ar-H)

\(^{13}\)C NMR (δ ppm, CDCl\(_3\)): 13.1, 17.6, 109.7, 116.1, 116.3, 125.4, 128.7, 128.9, 129.0, 129.2, 129.6, 130.1, 130.9, 132.5, 132.7, 135.0, 138.1, 138.5, 140.7, 149.6, 155.4, 155.8, 159.5, 162.8, 162.8, 165.3

Elemental Analysis
Actual: C, 65.87; H, 3.95; N, 16.46%
Found: C, 65.85; H, 3.92; N, 16.45%

7-(2,4-difluorophenyl)-5-(5-((4-fluorophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-3-methyl-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula: C_{28}H_{19}F_{3}N_{6}S
Molecular Weight (g.mol\(^{-1}\)): 528.5
Melting Point (°C): 199-201

FT-IR \( \nu_{\text{max}} \) cm\(^{-1}\) (KBr): 3112, 1662, 1580, 1493, 1394, 1258, 1224, 1095, 967, 847, 659

\(^1\)H NMR (δ ppm, DMSO-d\(_6\)): 2.34 (s, 3H, -CH\(_3\) of pyrazole), 2.94 (s, 3H, triazole-CH\(_3\)), 6.79 (m, 4H, Ar-H), 7.43 (m, 7H, Ar-H), 7.79 (s, 1H, Ar-H), 8.07 (m, 2H, Ar-H)

\(^{13}\)C NMR (δ ppm, DMSO-d\(_6\)): 13.1, 17.6, 109.3, 115.9, 116.4, 116.7, 125.6, 128.9, 129.0, 129.2, 129.5, 129.9, 130.3, 132.4, 132.7, 134.9, 137.3, 138.4, 140.9, 149.7, 155.2, 155.8, 159.7, 162.8, 165.3

Elemental Analysis
Actual: C, 63.63; H, 3.62; N, 15.90%
Found: C, 63.65; H, 3.64; N, 15.91%
5-((4-bromophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl
-7-(4-fluorophenyl)[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula: C_{27}H_{14}BrF_{n}N_{6}S
Molecular Weight (gm.mol⁻¹): 557.4
Melting Point (°C): 204-206

FT-IR: \( \nu_{\text{max}} \) cm⁻¹ (KBr): 3100, 1661, 1581, 1478, 1392, 1278, 1216, 1090, 997, 839, 689

\(^1^H\) NMR (δ ppm, CDCl₃): 2.41 (s, 3H, -CH₃ of pyrazole), 6.76 (d, 2H, Ar-H), 6.83 (m, 2H, Ar-H), 7.20 (m, 2H, Ar-H), 7.44 (m, 6H, Ar-H), 8.01 (m, 2H, Ar-H), 8.51 (s, 1H, triazole-H)

\(^1^3^C\) NMR (δ ppm, CDCl₃): 13.2, 109.4, 115.8, 116.2, 125.6, 128.0, 128.9, 129.1, 130.1, 130.3, 131.4, 131.6, 134.7, 138.3, 140.8, 149.3, 155.1, 155.8, 158.8, 162.9, 165.4

Elemental Analysis:
- Actual: C, 58.18; H, 3.25; N, 15.08%
- Found: C, 58.19; H, 3.24; N, 15.10%

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5-((4-bromophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl
-7-(2,4-difluorophenyl)[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula: C_{27}H_{12}BrF_{2}N_{6}S
Molecular Weight (gm.mol⁻¹): 575.4
Melting Point (°C): 196-198

FT-IR: \( \nu_{\text{max}} \) cm⁻¹ (KBr): 3108, 1665, 1596, 1476, 1391, 1280, 1231, 1094, 990, 849, 762, 695

\(^1^H\) NMR (δ ppm, DMSO-d₆): 2.35 (s, 3H, -CH₃ of pyrazole), 6.79 (d, 2H, Ar-H), 6.99 (m, 3H, Ar-H), 7.41 (m, 2H, Ar-H), 7.49 (m, 2H, Ar-H), 7.89 (s, 2H, Ar-H), 8.12 (m, 2H, Ar-H), 8.73 (s, 1H, triazole-H)

\(^1^3^C\) NMR (δ ppm, DMSO-d₆): 13.2, 116.3, 116.7, 116.9, 125.7, 127.5, 127.9, 128.9, 129.3, 129.8, 130.3, 132.7, 133.0, 133.8, 138.6, 140.9, 149.7, 155.3, 156.0, 158.8, 160.4, 162.9, 165.4

Elemental Analysis:
- Actual: C, 56.36; H, 2.98; N, 14.60%
- Found: C, 56.35; H, 2.97; N, 14.62%
5-(5-((4-bromophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl) -7-(4-fluorophenyl)-3-methyl-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula: C_{29}H_{20}BrFN_6S
Molecular Weight (g/mol): 571.4
Melting Point (°C): 203-205

FT-IR $\nu_{\text{max}}$ cm$^{-1}$ (KBr): 3113, 1659, 1588, 1475, 1390, 1280, 1220, 1097, 995, 840, 691

$^1$H NMR (δ ppm, CDCl$_3$): 2.35 (s, 3H, -CH$_3$ of pyrazole), 2.95 (s, 3H, triazole-CH$_3$), 6.79 (m, 5H, Ar-H), 7.50 (m, 4H, Ar-H), 7.88 (m, 3H, Ar-H), 8.07 (m, 2H, Ar-H)

$^{13}$C NMR (δ ppm, CDCl$_3$): 13.2, 17.7, 109.8, 116.2, 116.5, 125.6, 128.8, 129.0, 129.3, 129.5, 129.8, 130.2, 131.0, 132.7, 133.0, 135.1, 138.2, 138.5, 140.8, 149.7, 155.6, 155.9, 162.9, 165.4

Elemental Analysis
Actual: C, 58.85; H, 3.53; N, 14.71%
Found: C, 58.87; H, 3.50; N, 14.72%

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5-(5-((4-bromophenyl)thio)-3-methyl-1-phenyl-1H-pyrazol-4-yl) -7-(2,4-difluorophenyl)-3-methyl-[1,2,4]triazolo[4,3-a]pyrimidine

Molecular Formula: C_{29}H_{16}BrF$_2$N$_6$S
Molecular Weight (g/mol): 589.4
Melting Point (°C): 208-210

FT-IR $\nu_{\text{max}}$ cm$^{-1}$ (KBr): 3110, 1660, 1596, 1472, 1391, 1284, 1226, 1096, 992, 847, 761, 693

$^1$H NMR (δ ppm, CDCl$_3$): 2.59 (s, 3H, -CH$_3$ of pyrazole), 2.94 (s, 3H, triazole-CH$_3$), 6.80-6.97 (m, 3H, Ar-H), 6.99 (t, 1H, Ar-H), 7.28 (t, 2H, Ar-H), 7.39 (m, 6H, Ar-H), 7.94 (s, 1H, triazole-H)

$^{13}$C NMR (δ ppm, CDCl$_3$): 12.3, 27.6, 27.8, 31.5, 41.0, 49.1, 109.1, 111.0, 116.9, 117.2, 121.6, 125.0, 125.2, 126.7, 127.5, 127.7, 128.6, 128.8, 130.2, 131.5, 132.2, 132.3, 132.8, 134.7, 138.8, 147.8, 150.4, 151.0, 161.6, 163.8, 171.1, 194.8

Elemental Analysis
Actual: C, 57.05; H, 3.25; N, 14.26%
Found: C, 57.03; H, 3.26; N, 14.25%
5-(phenylthio)substituted triazolo[4,3-a]pyrimidine

Figure 5.2 Mass spectrum of compound 6a

Figure 5.3 FT-IR spectrum of compound 6a
5-(phenylthio)substituted triazolo[4,3-a]pyrimidine

Figure 5.4 $^1$H NMR spectrum of compound 6a

Figure 5.5 $^{13}$C NMR spectrum of compound 6a
5-(phenylthio)substituted triazolo[4,3-a]pyrimidine

Figure 5.6 Mass spectrum of compound 6h

Figure 5.7 FT-IR spectrum of compound 6h
$5$-(phenylthio)substituted triazolo[4,3-a]pyrimidine

Figure 5.8 $^1$H NMR spectrum of compound 6h

Figure 5.9 $^{13}$C NMR spectrum of compound 6h
5-(phenylthio)substituted triazolo[4,3-a]pyrimidine

Figure 5.10 Mass spectum of compound 6m

Figure 5.11 FT-IR spectrum of compound 6m
Figure 5.12 $^1$H NMR spectrum of compound 6m

Figure 5.13 $^{13}$C NMR spectrum of compound 6m
Figure 5.14 Mass spectrum of compound 6t

Figure 5.15 FT-IR spectrum of compound 6t
5-(phenylthio)substituted triazolo[4,3-a]pyrimidine

Figure 5.16 $^{1}$H NMR spectrum of compound 6t

Figure 5.17 $^{13}$C NMR spectrum of compound 6t
5.7 Biological study

The synthesized titled compounds were further evaluated for in vitro antimalarial, antioxidant, antituberculosis and antimicrobial potencies. The results corresponding to various tests are described below.

5.7.1 Antibacterial activity

In vitro antibacterial activity of 6a-t was performed using three gram +ve and three gram -ve bacteria using standard drugs according to NCCLS (National Committee for Clinical Laboratory Standards)[32]. The antimicrobial screening result data is mentioned in Table 5.1.

Almost all the compounds exhibited excellent resistance against bacteria in comparison to one of the standard drugs i.e. ampicillin or norfloxacin. It was observed that amongst Gram positive bacterial species compound 6o was reported to be maximum potent i.e. 62.5 µg/mL, against S. pneumoniae, in comparison to chloramphenicol i.e. 50 µg/mL. Compounds 6c, 6h, 6r, 6p and 6j display equivalent resistance as ampicillin i.e.100 µg/mL. Upon analyzing the results, compounds 6p, 6n and 6o show maximum or equipotency to that of five gram negative and positive bacterial species as that of ampicillin, norfloxacin and chloramphenicol. Compound 6r expressed better activity i.e.62.5 µg/mL against B. subtilis than chloramphenicol and ciprofloxacin. In gram negative bacterial screening, compound 6c and 6p exhibited brilliant potency as of chloramphenicol against S. Typhi and E. Coli respectively. Compounds 6f, 6j, 6l, 6m and 6r possessed prominent potency against four gram negative and positive bacterial species. For gram positive bacteria, compounds 6c, 6p, 6j, 6h and 6r expressed similar potency against S. Pneumoniae as that of ampicillin. Also in this case 6r, 6i, 6j 6o and 6a showed equipotency as that of ciprofloxacin especially against C. Tetani.
Table 5.1 *In vitro* antimicrobial activity expressed in terms of MIC, µg/mL of prepared derivatives 6a-t.

<table>
<thead>
<tr>
<th>Comp. code</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
<th>Fungi</th>
</tr>
</thead>
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<tr>
<td></td>
<td>S.P. MTCC</td>
<td>B.S. MTCC</td>
<td>C.T. MTCC</td>
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<tr>
<td>6a</td>
<td>250</td>
<td>250</td>
<td>125</td>
</tr>
<tr>
<td>6b</td>
<td>500</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>6c</td>
<td>100</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>6d</td>
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<td>500</td>
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</tr>
<tr>
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</tr>
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<td>n. t.</td>
<td>n. t.</td>
</tr>
<tr>
<td>F</td>
<td>n. t.</td>
<td>n. t.</td>
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</tr>
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</table>


It has been observed that against gram negative bacteria *E. Coli*, two compounds 6j and 6p were having excellent potency *i.e.* MIC 62.5µg/mL in comparison to chloramphenicol *i.e.*50µg/mL and ampicillin *i.e.*100µg/mL. Compounds 6k, 6e, 6g, 6b, 6i, 6n, 6o expressed the equipotency or somewhat less potency against *E. Coli* as that of ampicillin. Compounds 6a, 6h, 6i, 6l, 6n and 6t were observed to have equivalent potency against *S. Typhi* as ampicillin (*i.e.*100 µg/mL). Compounds 6d, 6f, 6m, 6r and 6t exhibited similar MIC as ampicillin against *V. Cholerae*. Remaining all compounds of the series possessed reasonable or lower activity against all gram negative and positive bacteria.
5.7.2 Fungicidal activity

The data of antifungal screening (Table 5.1) of synthesized pyrazole based triazolopyrimidine derivatives showed that some of the molecules exhibited excellent antifungal activity particularly against fungus *Candida albicans*. Compounds 6r, 6o and 6i were relatively more potent i.e. 250 µg/mL than griseofulvin. Compounds 6p, 6c, 6r, 6l, 6a, 6e and 6t expressed griseofulvin equivalent power i.e. 500 µg/mL. The compounds from the prepared series did not show almost similar or more potency against *A. Fumigatus*.

5.7.3 Antituberculosis activity

*In vitro* antituberculosis potency of compounds 6a-r was carried out at two different concentration (i.e. 250 and 100 µg/mL) against *M. tuberculosis* H37Rv strain as per standard procedure described by A. Rattan[33] using standard drugs. The screening data in form of % inhibition are mentioned in Table 5.2.

**Table 5.2** *In vitro* antituberculosis activity (% inhibition) of titled compounds 6a-t against M. tuberculosis H37Rv (at concentration 250 and 100µg/mL).

<table>
<thead>
<tr>
<th>Comp. code</th>
<th>% Inhibition 250 µg/mL</th>
<th>% Inhibition 100 µg/mL</th>
<th>Comp. code</th>
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<th>% Inhibition 250 µg/mL</th>
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<td>6l</td>
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<td>56</td>
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<td>6m</td>
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<td>33</td>
<td>6n</td>
<td>70</td>
<td>63</td>
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<td>28</td>
<td>22</td>
<td>6o</td>
<td>84</td>
<td>72</td>
</tr>
<tr>
<td>6e</td>
<td>53</td>
<td>41</td>
<td>6p</td>
<td>68</td>
<td>54</td>
</tr>
<tr>
<td>6f</td>
<td>91</td>
<td>82</td>
<td>6q</td>
<td>88</td>
<td>75</td>
</tr>
<tr>
<td>6g</td>
<td>78</td>
<td>64</td>
<td>6r</td>
<td>67</td>
<td>58</td>
</tr>
<tr>
<td>6h</td>
<td>66</td>
<td>57</td>
<td>6s</td>
<td>73</td>
<td>62</td>
</tr>
<tr>
<td>6i</td>
<td>53</td>
<td>39</td>
<td>6t</td>
<td>97</td>
<td>89</td>
</tr>
<tr>
<td>6j</td>
<td>71</td>
<td>59</td>
<td>Rifampicin</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>6k</td>
<td>85</td>
<td>73</td>
<td>Isoniazid</td>
<td>99</td>
<td>99</td>
</tr>
</tbody>
</table>

From the preliminary tubercular observation, compound 6m, 6f and 6t expressed highest *M. Tuberculosis* H37Rv strain bacterial resistance with growth inhibition ranging between 90-100% at concentration level 250µg/mL. Whereas compounds 6k, 6o and 6q exhibited growth inhibitions range between 80-90% at the same concentration level. The compounds showing superior growth inhibition power were
further tested at concentration level 100µg/mL. Remaining other compounds exhibited moderate % growth inhibition against *M. tuberculosis* H37Rv strain.

5.7.4 Antimalarial activity

*In vitro* anti-malarial potency of compounds 6a-r was carried out against *P. falciparum* strain using standard drugs. The screening data in the form 50% inhibitory concentration (*i.e.* IC$_{50}$) of parasitic growth is mentioned in Table 5.3.

**Table 5.3** *In vitro* antimalarial activity of titled derivatives 6a-t.

<table>
<thead>
<tr>
<th>Comp. code</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>Comp. code</th>
<th>IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>0.072</td>
<td>6l</td>
<td>0.067</td>
</tr>
<tr>
<td>6b</td>
<td>0.95</td>
<td>6m</td>
<td>1.35</td>
</tr>
<tr>
<td>6c</td>
<td>1.07</td>
<td>6n</td>
<td><strong>0.040</strong></td>
</tr>
<tr>
<td>6d</td>
<td>0.89</td>
<td>6o</td>
<td>0.96</td>
</tr>
<tr>
<td>6e</td>
<td>0.77</td>
<td>6p</td>
<td><strong>0.054</strong></td>
</tr>
<tr>
<td>6f</td>
<td>0.65</td>
<td>6q</td>
<td>0.78</td>
</tr>
<tr>
<td>6g</td>
<td>0.43</td>
<td>6r</td>
<td>0.82</td>
</tr>
<tr>
<td>6h</td>
<td>1.13</td>
<td>6s</td>
<td><strong>0.063</strong></td>
</tr>
<tr>
<td>6i</td>
<td>0.81</td>
<td>6t</td>
<td>1.03</td>
</tr>
<tr>
<td>6j</td>
<td>0.38</td>
<td>Chloroquine</td>
<td>0.020</td>
</tr>
<tr>
<td>6k</td>
<td>0.58</td>
<td>Quinine</td>
<td>0.268</td>
</tr>
</tbody>
</table>

Duplicate runs were performed for each experiment and average values of IC$_{50}$ are depicted in Table 5.3. Compounds 6p, 6l, 6n, 6a and 6s were having IC$_{50}$ ranging between 0.040 to 0.072 for *P. falciparum* strain, which displayed enhanced activity as compared to quinine (IC$_{50}$ 0.268). Remaining other compounds were less active than chloroquine and quinine against *P. falciparum*. 
5.7.5 Antioxidant activity

Table 5.4 *In vitro* antioxidant activity of compounds 6a-t.

<table>
<thead>
<tr>
<th>Comp. code</th>
<th>OD (593 nm)</th>
<th>FRAP value(^a)</th>
<th>Comp. code</th>
<th>OD (593 nm)</th>
<th>FRAP value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>1.703</td>
<td>343.63</td>
<td>6l</td>
<td>1.156</td>
<td>233.25</td>
</tr>
<tr>
<td>6b</td>
<td>0.560</td>
<td>112.99</td>
<td>6m</td>
<td>2.415</td>
<td>487.29</td>
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<tr>
<td>6c</td>
<td>1.175</td>
<td>237.09</td>
<td>6n</td>
<td>2.175</td>
<td>438.87</td>
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<tr>
<td>6d</td>
<td>0.967</td>
<td>195.12</td>
<td>6o</td>
<td>2.213</td>
<td>446.53</td>
</tr>
<tr>
<td>6e</td>
<td>1.115</td>
<td>224.98</td>
<td>6p</td>
<td>1.127</td>
<td>227.40</td>
</tr>
<tr>
<td>6f</td>
<td>1.213</td>
<td>244.75</td>
<td>6q</td>
<td>1.288</td>
<td>259.89</td>
</tr>
<tr>
<td>6g</td>
<td>1.604</td>
<td>323.65</td>
<td>6r</td>
<td>1.230</td>
<td>248.18</td>
</tr>
<tr>
<td>6h</td>
<td>1.450</td>
<td>292.58</td>
<td>6s</td>
<td>2.273</td>
<td>458.64</td>
</tr>
<tr>
<td>6i</td>
<td>2.143</td>
<td>432.41</td>
<td>6t</td>
<td>0.983</td>
<td>198.34</td>
</tr>
<tr>
<td>6j</td>
<td>1.894</td>
<td>382.17</td>
<td>A.A.</td>
<td>2.501</td>
<td></td>
</tr>
<tr>
<td>6k</td>
<td>0.830</td>
<td>167.47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) A.A. mmol per 100 g of the sample. FRAP = ferric reducing antioxidant power

Upon investigation of the FRAP (Ferric Reducing Antioxidant Power), *(Table 5.4)* compounds 6a-t showed FRAP values ranging between 467.52 to 75.46 mmol per 100 g of compounds. This indicates that compounds 6a-t are better antioxidants. Amongst them compound 6m expressed remarkable antioxidant activity.
5.8 Structure-activity relationship (SAR)

The result of the pharmacological screening is helpful in correlating the diverse potency of compounds and pattern of substitution on thiophenol and acetophenone moiety. From the structure activity relationship, it is revealed that, electron attracting groups such as fluorine and fluorine containing compounds showed the diversified importance to boost antimicrobial potency in compounds covered under the study (Figure 5.2).

5.9 Conclusion

We have designed, synthesized, characterized and screened 5-(phenylthio)-substituted-triazolo[4,3-a]pyrimidine derivatives for their diversified antimalarial, antioxidant, antituberculosis and antimicrobial potencies. The biological screening of synthesized compounds revealed importance of the series. Compounds 6a, 6l, 6n, 6p and 6s were observed to possess noteworthy antimalarial potency (i.e. IC$_{50}$) as compared to quinine. Amongst the prepared library, compound 6m possessed remarkable antioxidant potency. Compounds 6t, 6m and 6f revealed excellent tuberculosis resistance with growth inhibition range between 90-100% at concentration level i.e. 250µg/mL. Many of the synthesized compounds exhibited excellent antibacterial potency.
References:

[21] M. Kumar, V. Kumar, V. Beniwal, Synthesis of some pyrazolylaldehyde N-isonicotinoyl hydrazones and 2, 5-disubstituted 1, 3, 4-oxadiazoles as DNA photocleaving agents, Medicinal Chemistry Research, 24 (2015) 2862-2870.


