ABSTRACT

The reaction of anthranilic acid with acetic anhydride produced acetanthranil. Further reacting the product with aromatic amines and aromatic aldehydes respectively produces a series of 2, 3-disubstituted-4(3H)-quinazolinone derivative. Compounds IVa-f were synthesized to investigate their anti-malarial activities. The in vivo anti-malarial activity of these compounds was tested on P. berghei infected mice at two doses (48.46 μmol/kg/day and 96.92 μmol/kg/day). Most active compounds IVa (64.02%), IVc (77.25%) and IVe (73.54%) showed a dose dependent increase in anti-malarial activities. Invivo acute toxicity studies of these compounds IVa-f indicates all to be non-toxic and well tolerated by the experimental animals up to 300 mg/ kg administered orally and 140mg/ kg administered parenterally. Therefore, test compounds IVa-f would represent a successful lead compound for the development of new class for anti-malarial agents.

KEYWORDS: 4(3H)-quinazolinone, in vivo, anti-malarial, in vivo acute toxicity.

INTRODUCTION

Malaria is one of the most prevalent parasitic infections in the world and certainly the most detrimental. Each year, over two million people die from the disease, with the vast majority
of the deaths in children under five years old in Sub-Saharan Africa.\textsuperscript{[1]} According to the World Health Organization’s World Malaria Report 2009 and the Global Malaria Action Plan, 3.3 billion people (half the world’s population) live in areas at risk of malaria transmission in 109 countries and territories. Out of these 109 countries, 35 (30 in sub-Saharan Africa and 5 in Asia) account for 98% of global malaria deaths.\textsuperscript{[2]}

In humans, one or more of four species of intracellular protozoan parasites belonging to the genus \textit{Plasmodium}; \textit{P. falciparum}, \textit{P. malarae}, \textit{P. vivax} and \textit{P. ovalae} cause malaria infection in human. \textit{P. falciparum} malaria receives special attention because it is the major cause of death.\textsuperscript{[3]}

Resistance to anti-malarial drugs has been observed in \textit{P. falciparum} and \textit{P. vivax}. \textit{P. falciparum} has developed resistance to nearly all anti-malarial drugs in current use.\textsuperscript{[4]}

Moreover, according to research report in 2008, the now days widely used drug, artemisinin is losing its potency in Cambodia and increased efforts are required to prevent drug-resistant malaria from spreading across the globe.\textsuperscript{[5]} This finding was subsequently supported by a detailed study in 2009 from Western Cambodia.\textsuperscript{[6]} Hence novel strategies to circumvent resistance are needed. A growing body of evidence supports the role for drugs able to restore susceptibility to traditionally efficacious compounds like chloroquine.\textsuperscript{[7]}

In recent years there has been an increasing interest in the chemistry of 4(3\textit{H})-quinazolinones because of their biological importance.\textsuperscript{[8]} Quinazoline is a bicyclic compound consisting of a pyrimidine system fused at 5, 6 with benzene ring having broad spectrum of medicinal values such as anti bacteria, anti fungal, anti cancer, anti-inflammatory, antiviral, anti tuberculosis, CNS depressant activity, Anti-parkinsonism, bronchodilator activity etc.\textsuperscript{[9]} Quinazolinone nucleus is found in many bioactive natural products. So, because of these reasons much attention is being paid for the synthesis of quinazolinone derivatives.\textsuperscript{[10]}

Quinazolinone are considered as a privileged scaffold in drug discovery and drug development. Among the two isomers of quinazolinone, 4-(3\textit{H})-Quinazolinone being more common show various biological activities and prove its major application in the field of medicine.\textsuperscript{[11]} Thus, in this work also 4-(3\textit{H})-quinazolinones are evaluated for their antimalarial activities \textit{invivo} in an effort to produce potential lead compounds.
EXPERIMENTAL

Materials
Melting points were determined in open capillaries using Buchi (B-540) melting point apparatus and are uncorrected. IR spectra were recorded on a SHIMADZU 8400SP FT-IR spectrophotometer. $^1$H NMR spectral data were recorded on Bruker Avance DMX400 FT-NMR spectrometer using TMS as an internal reference (chemical shifts are given in $\delta$, ppm). Elemental microanalyses were done on Perkin Elmer 2400 elemental analyzer at Micro analytical unit analyzer; all of the new compounds were analyzed for C, H, and N and agreed with the proposed structures within $\pm$0.4% of the theoretical values. The progress of the reaction and purity of compounds were monitored by TLC analytical silica gel plates of 0.25 mm thickness. The spots were visualized using iodine vapor. All the reagents used were AR grade.

General procedure for the synthesis of the compounds (3-aryl-2-(substituted styryl)-4(3H)-quinazolinones) IVa-f
A solution of anthranilic acid I (0.145 mol) with excess acetic anhydride was heated under reflux for 1 hr where upon a solid mass of acetanthranil II was obtained. A mixture of acetanthranil II, (0.1 mol) and an equimolar amount of the appropriate aromatic amine was then heated under reflux for 5-7 hrs. The compounds formed IIIa-d were cooled and recrystallized from ethanol. An equimolar amount of 3-Aryl-2-methyl-4(3H)-quinazolinone IIIa-d, (10 mmol) and the appropriate aromatic aldehyde was further reacted in the presence of anhydrous zinc chloride by heating under reflux for 10-12 hrs.

(E)-3-Phenyl-2-[2-(pyridin-4-yl) vinyl]-4(3H)-quinazolinone IVa
Yield IVa 71.2%; m.p. 250-252°C; IR (Nujol) (cm$^{-1}$): 1680 (C=O) and 1593 (C=N); $^1$H NMR (CDCl$_3$/CCl$_4$) ppm: 6.55 (d, 1H, J = 15.53Hz, vinyl-C$_2$ H), 7.16 (d, 2H, J = 5.29 Hz, pyridin-C$_2$,6 H), 7.35 (d, 2H, J = 6.38Hz, phenyl-C$_3$,5 H), 7.55 (t, 1H, J = 8.08Hz, quinazolin-C$_7$ H), 7.58-7.67 (m, 3H, phenyl-C$_2$,4,6 H), 7.78-7.86 (m, 2H, quinazolin-C$_6$,8 H), 7.90 (d, 1H, J = 15.53Hz, vinyl-C$_1$ H), 8.32 (dd, 1H, J$_1$ = 0.490Hz, J$_2$ = 1.407Hz quinazolin-C$_5$ H) and 8.57 (d, 2H, J = 5.29 Hz, phenyl-C$_3$,5 H); Anal. Calcd for C$_{21}$H$_{15}$N$_3$O: C, 77.52; H, 4.65; N, 12.91. Found: C, 77.21; H, 4.82; N, 13.13.

(E)-2-[2-(Pyridin-4-yl) vinyl]-3-p-tolyl-4(3H)-quinazolinone IVb
Yield IVb 78.0%; m.p. 223-225°C; IR (Nujol) (cm$^{-1}$): 1682 (C=O) and 1614 (C=N); $^1$H NMR (DMSO-d$_6$) ppm: 2.4 (s, 3H, p-tolyl CH$_3$), 6.60 (d, 1H, J = 15.70Hz, vinyl-C$_2$ H), 7.30-7.45
(E)-2-(4-hydroxy-3-methoxystyryl)-3-phenyl-4(3H)-quinazolinone IVc
Yield IVc 63.4%; m.p. 260-262°C; IR (Nujol) (cm⁻¹): 1673 (C=O); 1637 (C≡N); 1120 and 1206 (C-O-C); 1H NMR (CDCl₃/CDCl₄) ppm: 3.8 (s, 3H, -O-CH₃), 6.25 (d, 1H, J = 15.69Hz, vinyl-C₂H), 6.85-7.0 (m, 3H, 4-hydroxy-3-methoxyphenyl-C₂,5,6 H), 7.35 (d, 2H, J = 7.84Hz, phenyl-C₃,₅ H), 7.50 (t, 1H, J = 7.79Hz, quinazolin-C₇ H), 7.55-7.65 (m, 3H, phenyl-C₂,₄,₆ H), 7.80 (m, 2H, quinazolin-C₆,₈ H), 7.92 (d, 1H, J = 15.69Hz, vinyl-C₁ H), 8.30 (dd, 1H, J₁ = 0.510Hz, J₂ = 1.430Hz quinazolin-C₃ H); Anal. Calcd for C₂₃H₁₈N₂O₃: C, 74.58; H, 4.90; N, 7.56. Found: C, 74.27; H, 5.22; N, 7.81.

(E)-2-[2-(Pyridin-4-yl)-vinyl]-3-o-tolyl-4(3H)-quinazolinone IVd
Yield IVd 59.5%; m.p. 224-226°C; IR (Nujol) (cm⁻¹): 1652 (C=O) and 1634 (C≡N); 1H NMR (DMSO-d₆) ppm: 2.4 (s, 3H, CH₃), 6.60 (d, 1H, J = 15.63Hz, vinyl-C₂ H), 7.30-7.45 (m, 6H, tolyl-C₃,₄,₅,₆ H and pyridin-C₂,₆ H), 7.60 (t, 1H, J = 7.73Hz, quinazolin-C₇ H), 7.75-7.85 (m, 2H, Vinyl-C₁ H and quinazolin-C₈ H), 7.90 (t, 1H, J = 7.34Hz, quinazolin-C₆ H), 8.15 (dd, 1H, J₁ = 0.415Hz, J₂ = 1.390Hz quinazolin-C₅ H) and 8.55 (d, 1H, J = 5.32Hz, pyridin-C₃,₅ H); Anal. Calcd for C₂₂H₁₇N₃O: C, 77.86; H, 5.05; N, 12.38. Found: C, 78.08; H, 4.79; N, 12.54.

(E)-2-[2-(Pyridin-4-yl)-vinyl]-3-phenylamine-4(3H)-quinazolinone IVe
Yield IVe 67.5%; m.p. 264-266°C; IR (Nujol) cm⁻¹: 3250 (N-H); 1700 (C=O) and 1625 (C≡N); 1H NMR (DMSO-d₆) ppm: 6.70 (d, 2H, J = 8.39Hz, phenyl-C₃,₆ H), 6.85 (t, 1H, J = 7.36Hz, 3-phenylamine-C₄ H), 7.23 (t, 2H, J = 8.34Hz, 3-phenylamine-C₅,₇ H), 7.58 (t, 1H, J = 7.61Hz, quinazolin-C₇ H), 7.62 (d,2H, J = 5.31Hz, pyridin-C₂,₆ H), 7.67 (d, 1H, J = 15.90Hz, vinyl-C₂ H), 7.82 (d, 1H, J = 8.27Hz, quinazolin-C₈ H), 7.90 (t, 1H, J = 8.30Hz, quinazolin-C₆ H), 8.00 (d, 1H, J = 15.90Hz, vinyl- C₁ H), 8.15 (dd, 1H, J₁ = 0.450Hz, J₂ = 0.976Hz, quinazolin-C₅ H), 8.60 (d, 2H, pyridin-C₃,₅ H) and 9.25 (s, 1H, 3-phenylamine N-H); Anal. Calcd for C₂₁H₁₆N₄O: C, 74.10; H, 4.74; N, 16.46. Found: C, 74.36; H, 4.48; N, 16.24.
(1E,3E)-3-Phenyl-2-[4-phenylbut-1,3-dienyl]-4(3H)-quinazolinone IVf

Yield IVf 85.0%; m.p. 228-230°C; IR (Nujol) (cm⁻¹): 1679 (C=O) and 1625 (C=N); ¹HNMR (CDCl₃/CCl₄) ppm: 5.95 (d, 1H, J = 14.69Hz, butdiene-C₄H), 6.75 (t, 1H, J = 15.43Hz, butdiene-C₂H), 6.95 (d, 1H, J = 15.43Hz, butdiene-C₁H), 7.25-7.35 (m, 5H, butdiene-C₃H), quinazolin-C₆,₈ H and phenyl-C₃,₅ H), 7.73-7.88 (m, 3H, quinazolin-C₇H and phenyl C₃,₂,₆ H), 7.40-7.70 (m, 6H, phenyl-C₃ H and but-phenyl-C₂,₃,₄,₅,₆ H) and 8.3 (dd, 1H, J₁ = 0.897Hz, J₂ = 1.380Hz quinazolin-C₅ H); Anal. Calcd for C₂₄H₁₈N₂O: C, 82.26; H, 5.18; N, 7.99. Found: C, 82.46; H, 4.88; N, 8.08.

Biological Assays
Experimental animals
Swiss albino mice of both sexes, weighing 25-35 g and aged 6-8 weeks purchased from Ethiopian Health and Nutrition Institute were used in the study. The mice were acclimatized to the laboratory conditions (temperature of 23-25°C with average relative humidity of 60%) for a period of 7 days before use. The mice were housed in standard cages and maintained on standard pelleted diet and water.

Test strains
The rodent malaria parasite, P.berghei ANKA strain was obtained from the Bio-medical laboratory, Department of Biology, Faculty of Science, Addis Ababa University and used to infect the mice for a four-day suppressive test.

In vivo anti-malarial activity
A four-day suppressive standard test was used to evaluate the in vivo anti-malarial activities of the synthesized compounds, IVa-f. P. berghei were maintained in the laboratory mouse through infected blood transfusion. Mice were allowed to acclimatize to the laboratory environment under room temperature for a week before being used for the experiments. Each experimental animal was given with inoculations of 0.2 ml (about 2x10⁷ parasites) intraperitoneal on day zero, which is expected to produce a steadily rising infection. After 2 hrs, the infected mice were weighed and randomly divided into eight groups of five mice per group per cage. Group 1 received a vehicle containing 7% Tween 80 and 3% ethanol in distilled water that served as a negative control. Group 2 that served as positive control was given 25 mg/kg/day (0.04846 mmol/kg/day) of the standard drug, chloroquine phosphate (Mwt = 515.86 gram/mol). Groups 3, 4, 5, 6, 7 and 8 were treated with equimolar amounts of...
the synthesized compounds (0.04846 mmol/kg/day) that was dissolved in 7% Tween 80 and 3% ethanol through oral route.\cite{19, 20}

The above treatment was continued over 4-days. Twenty four hours after the last treatment (5\textsuperscript{th} day), blood smears were prepared from the tail of all mice, air dried, fixed with absolute methanol & stained with 6% Giemsa. The parasitemia was then determined microscopically by counting 4 fields of approximately 100 erythrocytes per field.

The efficacies of compounds were finally assessed by comparison of blood parasitemia and mouse survival time in treated and untreated mice. The 4-day standard suppressive test was repeated for the second time for all the compounds at a dose level of twice the amount in the first dose (0.09692 mmol/kg/day).

\textit{In vivo} acute toxicity test

The oral acute toxicity of compounds was investigated using male Swiss albino mice (20g each, Medical Research Institute, Alexandria University) according to reported methods.\cite{16} The mice were divided into groups of six mice each and fasted over night. The compounds were given orally, suspended in 1% gum acacia, in doses of 10, 50, 100, 200 and 300 mg/kg. After Oral administration of the target compounds, the mice were observed closely during 24 hrs with special attention to the first four hours. Additionally the test compounds were investigated for their parenteral acute toxicity in groups of six mice per cage. The compounds or their vehicle, propylene glycol (control) was given by intraperitoneal injection in doses of 20, 40, 80, 120, 140 mg/kg. The percentage survival was followed up to 7 days.\cite{16}

Acute toxicity signs like sedation, lacrimation, hair erection, blinking, urination, muscle weakness, sedation and convulsion, reduction in motor, diarrhea, sleep, coma and death were checked in the test mice.\cite{17}

\textbf{Statistical Analysis:} The results of the study were expressed as mean ± standard deviation and statistical significance for suppressive test was determined by one-way ANOVA using Microcal Origin 6.0 soft ware. Data on survival time, % parasitemia and % suppression was analyzed using Microsoft office excel 2007. All data was analyzed at 95% confidence interval (P=0.05).
RESULTS AND DISCUSSION

Chemistry
A solution of anthranilic acid I (20g, 0.145 moles) in acetic anhydride (23 ml) was heated under reflux for 1 hr. The excess acetic anhydride was then washed with anhydrous petroleum ether to obtain 1-benzoazin-4-one II, scheme 1. A mixture of cetanthranil II, (1.175 g, 7.5 mmole) and equimolar amounts of the appropriate aromatic amine or phenylhydrazine was heated under reflux at 190 °C for 5 hrs. The dark sticky mass formed was cooled and recrystallized from ethanol, scheme 1.

\[
\begin{align*}
\text{Ar}^1 & \quad \text{Ar}^2 \\
\text{I} & \quad \text{II} & \quad \text{III a-d} & \quad \text{Ar}^2-\text{CHO} & \quad \text{IVa-f}
\end{align*}
\]

Scheme 1: Synthesis of 2,3-disubstituted-4(3H)-quinazolinone derivatives IVa-f.

A solution of 3-aryl-2-methyl-4(3H)-quinazolinones III a-c, (10 mmole) and 3-arylamino-2-methyl-4(3H)-quinazolinones III d, (10 mmole) in acetic anhydride (10ml), was allowed to react with an equimolar amount of appropriate aldehyde in the presence of 10mg of anhydrous zinc chloride by heating under reflux for 10 hrs, scheme 1.

\[
\begin{align*}
\text{IVa} & \quad \text{IVb} & \quad \text{IVc} & \quad \text{IVd} & \quad \text{IVe} & \quad \text{IVf}
\end{align*}
\]

In vivo anti-malarial activity
The synthesized compounds were evaluated for their in vivo anti-malarial activity on, \textit{P. berghei} ANKA strain infected mice and the results are listed in Table 1 and 2. Chloroquine phosphate was used as positive control. The target compounds were used in two doses. The
initial dose was 48.46 μmol/kg/day of chloroquine phosphate and equimolar concentration of each of the synthesized compounds. The dose was doubled (96.92 μmol/kg/day) to investigate the anti-malarial effect is dose-dependent or not. The mean percent of parasitemia measured for all test compounds changed significantly from those in the negative control (P<0.05). This shows that the compounds are some protective effect, table 1.

Table 1: Anti-malarial activities of IVa-f at dose of 48.46 μmol/kg*

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Dose (mg/kg)</th>
<th>% Parasitaemia</th>
<th>% Suppression</th>
<th>Mean survival time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVa</td>
<td>15.77</td>
<td>33.67 ± 0.70</td>
<td>50.67</td>
<td>7.9 ± 0.40</td>
</tr>
<tr>
<td>IVb</td>
<td>16.45</td>
<td>46.00 ± 0.69</td>
<td>32.60</td>
<td>7.0 ± 0.20</td>
</tr>
<tr>
<td>IVc</td>
<td>17.95</td>
<td>17.75 ± 0.39</td>
<td>73.99</td>
<td>9.8 ± 0.50</td>
</tr>
<tr>
<td>IVd</td>
<td>16.45</td>
<td>45.50 ± 0.77</td>
<td>33.33</td>
<td>6.7 ± 0.34</td>
</tr>
<tr>
<td>IVe</td>
<td>16.49</td>
<td>22.75 ± 0.5</td>
<td>66.67</td>
<td>9.1± 1.12</td>
</tr>
<tr>
<td>IVf</td>
<td>16.98</td>
<td>27.67 ±0.29</td>
<td>59.46</td>
<td>8.2 ± 0.30</td>
</tr>
<tr>
<td>NC**</td>
<td>1ml/100 g</td>
<td>68.25 ± 0.73</td>
<td>0.0</td>
<td>6.1 ± 0.17</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>25</td>
<td>0.0</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are Mean ± SD, P<0.05, **NC: Negative control, ND: No death recorded over the experimental period.

Compound IVc was the most active as compared to other target compounds but less than that of positive control group that did not show any death during the experimental period, Table 1. This highest suppression effect of IVc can be attributed to the hydroxyl group (OH) at para position of 2-styryl group that forms hydrogen bonding with the receptor site. Compound IVe revealed the next better anti-malarial activity with percent suppression of 66.67% that is further confirmed by the mean survival time (9.1± 1.12), Table 1. This could be because of the presence of secondary amine group (N-H) on the 3-quinazolinone position that increases the binding strength by hydrogen bonding. Moreover, the planar shape of the phenyl group can be considered as a good in put in this regard. The mean percent suppression of IVd and IVb was not significantly different (p>0.05) at first dose showing that their activity is comparable but lower than that of IVa.

This could be due to the tetrahedral shape of the ortho or para methyl groups in the structure of compounds, IVd and IVb that hinders the strong hydrophobic interaction with the receptor pocket. The activity of compound IVf was less than that of IVc that could be due to the extension (increase in length) of the group at 3-quinazolinone position and lack of electronegative element.
Table 2: Anti-malarial activities of IVa-f at dose of 96.92 µmol/kg *

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Dose (mg/kg)</th>
<th>% Parasitemia</th>
<th>% Suppression</th>
<th>Mean survival time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVa</td>
<td>31.54</td>
<td>22.67 ± 0.58</td>
<td>64.02</td>
<td>8.7 ± 1.0</td>
</tr>
<tr>
<td>IVb</td>
<td>32.90</td>
<td>37.67 ± 0.72</td>
<td>40.21</td>
<td>7.4 ± 0.57</td>
</tr>
<tr>
<td>IVc</td>
<td>35.90</td>
<td>14.33 ± 0.40</td>
<td><strong>77.25</strong></td>
<td>10.0 ± 1.1</td>
</tr>
<tr>
<td>IVd</td>
<td>32.90</td>
<td>43.00 ± 0.26</td>
<td>31.75</td>
<td>7.0 ± 0.5</td>
</tr>
<tr>
<td>IVe</td>
<td>32.98</td>
<td>16.67 ± 0.25</td>
<td><strong>73.54</strong></td>
<td>9.5 ± 0.8</td>
</tr>
<tr>
<td>IVf</td>
<td>33.96</td>
<td>27.67 ± 0.47</td>
<td>56.08</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>NC**</td>
<td>1ml/100 g</td>
<td>63.00 ±0.40</td>
<td>0.0</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>Chloroquine Phosphate</td>
<td>25</td>
<td>0.0</td>
<td>100</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Values are Mean ± SD, P<0.05, **NC: Negative control. ND: No death recorded over the experimental period.

When the dose was doubled compounds; IVa, IVb, IVc and IVe showed a dose-dependent increase in mean percent suppression as clearly indicated in figure1. On the other hand, despite the fact that compound IVd had some suppressing effect, the mean percentage suppressions at the two doses was not significantly different at 95% confidence level(p>0.05). This indicates that the activity of this compound is not dose dependent. However, the mean percent suppression of compound IVf at the two dose levels was significantly different confirming that there is a decrease in the activity. Generally the increase in activity at lower dose of compounds IVd and IVf seems insignificant at the minimal dose in this experiment, 48.46 µmol/kg. Nevertheless, the compounds might show an inverse relation of dose and activity, has additional lower doses been administered.

Figure1: Comparative antimalarial activity of the two doses of compounds IVa-f
CONCLUSION
Several 2, 3-disubstituted-4(3H)-quinazolinone derivatives, IVa-f were synthesized to investigate their anti-malarial activity. The target compounds were obtained in a good yield (59.5-85%) by applying different chemical reactions like cyclization and condensation reactions. The structure of the final compounds was confirmed by using elemental microanalysis, IR and 1HNMR.

The in vivo anti-malarial activity of these compounds was tested on P.berghei infected mice at two doses (48.46 µmol/kg/day and 96.92 µmol/kg/day). Chloroquine phosphate was used as positive control. Compounds IVa (64.02%), IVc (77.25%) and IVe (73.54%) showed a dose dependent increase in anti-malarial activities, being the most active among all. Furthermore, acute toxicity test results indicated that all the compounds (IVa-f) proved to be non-toxic and well tolerated by the experimental animals up to 300 mg/ kg in oral and 140mg/ kg in parenteral studies. Therefore, the test compounds IVa-f would represent a rewarding matrix of lead compounds for the development of new class of anti-malarial agents that would deserve further investigation and derivatization.

RECOMMENDATION
As depicted in figure1, compounds IVa, IVb, IVc and IVe showed an increase in activity with increase in dose level given to the experimental animals. However, compounds IVd and IVf demonstrated a slight decrease in percentage suppression with increase in dose. Therefore, further study of these two compounds is needed in investigating their potential to be a candidate lead compound. Besides, further toxicity studies need to be done to prove the sub-acute and chronic safety of the most active compounds.

ACKNOWLEDGMENT
We are very grateful to our college staff members for unreserved guidance and constructive suggestions and comments from the stage of proposal development to this end. We would like to thank Addis Ababa University for supporting the budget which required for this research.

CONFLICT OF INTERESTS
The authors declare no conflict of interests.
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