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# UTILITY OF AMINO ACID COUPLED 1,2,4-TRIAZOLES IN ORGANIC SYNTHESIS: SYNTHESIS OF SOME NEW ANTILEISHMAINAL AGENTS

Ahmed M.M. El-Saghier, Mounir A.A. Mohamed*, Omayma A. Abdalla and Asmaa M. Kadry

Chemistry Department, Faculty of Science, Sohag University, Sohag, Egypt

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**ABSTRACT**. Starting from 3-amino-5-(2-hydroxyphenyl) amino acid coupled triazoles **1a-e**, new 3-(2-hydroxyphenyl)-*3H*-imidazo[2,1-c][1,2,4]triazol-6(*5H*)-one **2a,b**, **3b,d**, **6a** and 3-*N*-arly(alkyl) amino acid coupled triazoles **4b,d**, **7a,c,d,e** have been synthesized as potential antileishmanial agents. The structures of the newly synthesized compounds were confirmed using elemental and spectral analyses (FT-IR, $^{1}$H-NMR, $^{13}$C-NMR and MS). The *in vitro* antileishmanial potency of the synthesized compounds was evaluated compared to Amphotericin B deoxycholate and miltefosine as lead references. Compounds **2b, 7d** and **7e** showed perfect $IC_{50}$ values corresponding to amphotericin B and more patent than miltefosine against L. aethiopica promastigotes.

**KEY WORDS**: Amino acids, Coupled, Imidazo-1,2,4-Triazole, Promatigote, Antileishmanial

## INTRODUCTION

Leishmaniasis occurs as visceral, cutaneous, mucocutaneous and diffused mucocutaneous leishmaniasis and is caused by Leishmania genus and transmitted by bite of infected female sand fly [1]. Leishmania parasites have two basic life stages: an extracellular motile stage (promastigote) inside an invertebrate host and an intracellular non-motile stage (amastigote) inside a vertebrate host [2]. The common form of leishmaniasis is Cutaneous leishmaniasis (CL) which widely distributed all around the world [3]. Within the last decade, the treatment is limited to a few drugs, such as amphotericin B, miltefosine and paromomycin and far from satisfactory [4], although a broad array of species can be responsible to cause leishmaniasis, affecting humans and animals [6-11]. Miltefosine-resistant Leishmania donovani promastigotes also demonstrated modification in sterol biosynthesis and lipid compositions which influences the membrane fluidity and permeability and ultimately may affect drug-membrane interactions [5]. Other possible oral treatments for CL include azole antifungals that show *in vitro* [12] and *in vivo* activity against Leishmania [13–20]. Triazoles antifungals inhibit 14α-lanosterol demethylation, causing accumulation of 14α-methyl sterols blocking the synthesis of ergosterol, the main sterol of Leishmania such as fluconazole, eliminating promastigote and amastigote of Leishmania sp. as well as Trypanosoma cruzi, protozoa phylogenetically related to Leishmania [21].

In deeps, imidazo triazole moieties have been widely reported in the mainstream as well as in the patent literature [22-26], also demonstrated dual activity against leishmania [27, 28]. Therefore, as a part of our effort to use a simple and effective method for the synthesis of bioactive imidazo[1,2,4]triazole analogues searching for a new drug candidates for Leishmanial aethiopica promatigote.

## EXPERIMENTAL

Melting points were determined in open-glass capillaries using a Griffin melting point apparatus and are all uncorrected. Infrared spectra (IR) were recorded on Perkin Elmer 1430 infrared spectrophotometer. $^{1}$H-NMR and $^{13}$C-NMR spectra were scanned on Jeol-400 MHz NMR-

*Corresponding author. E-mail: mounir_abbas@yahoo.com

spectrometer (DMSO-$d_6$) and chemical shifts are given in δ (ppm) down field from tetramethylsilane (TMS) as internal standard. Micro analyses were performed on Vario El Fab-Nr elemental analyzer. Following up of the reactions as performed by thin-layer chromatography (TLC) on silica gel (60GF254) coated Glass plates and the spots were visualized by exposure to iodine Vapors or UV-lamp at 254 nm for few seconds.

*General procedures for synthesis of compounds **2a,b** and **3b,d***

*Method A.* To a solution of 2-amino-5(2-hydroxy phenyl)-1,2,4-triazole carboxylic acid **1a,b,d** (0.01 mol) and chloroacetyl chloride (1.1 g, 0.01 mol) in acetonitrile, 2 g of potassium carbonate in 1 mL water was added as a base. The reaction mixture was refluxed for 5 h to complete the reaction (monitored by TLC). After completion of the reaction, the reaction mixture was cooled to room temperature and the solid mass was filtered off, washed with water and recrystallized from ethanol.

*Method B.* To a solution of 2-amino-5(2-hydroxy phenyl)-1,2,4-triazole carboxylic acid **1b,d** (0.01 mol) and chloroacetyl chloride (1.1 g, 0.01 mol) in chloroform and triethylamine as a catalyst. The reaction refluxed for 7 h to complete the reaction (monitored by TLC). After completion of the reaction, mixture was cooled to room temperature and the solid mass was filtered off and recrystallized from ethanol to give compounds **3b,d**.

*3-(2-Hydroxyphenyl)-3H-imidazo[2,1-c][1,2,4]triazol-6(5H)-one* ***2a***. Anal. calcd. for $C_{10}H_8N_4O_2$ (Mr = 216.20): C, 55.50; H, 3.70; N, 25.90. Found: C, 55.17; H, 3.39; N, 25.50. IR (ν, $cm^{-1}$); 3404 (broad O–H), 3038 (C–H) aromatic, 2991 (C–H) aliphatic, 1695 (C=O), 1590 (C=N). $^1$H-NMR (400 MHz; $d_6$-DMSO, δ ppm); 3.92 (s, 2H, $CH_2$), 3.99 (s, 1H, CH-triazole), 6.89-7.89 (m, 4H, ArH), 11.31 (s, 1H, OH, disappeared by $D_2O$), $^{13}$CNMR : 38.62 ($CH_2$), 74.17 (CH-triazole), 114.95, 118.78, 126.58. 128.78, 134.66, 143.05 (ArC), 158.22 (C=N), 170.10 (C=O). MS (m/$z^+$, %): 215.20 ($M^{-2}$, 0.1); 194.10 (100); 167.10 (21.12); 149.15 (24.99), 133.15 (41.56); 120.10 (30.79); 91.10 (71.73); 65.05 (50.65).

*3-(2-Hydroxyphenyl)-3-methyl-3H-imidazo[2,1-c][1,2,4]triazol-6(5H)-one* ***2b***. Anal. calcd. for $C_{11}H_{10}N_4O_2$ (Mr = 230.24): C, 52.56; H, 4.34; N, 24.32. Found: C, 52.26; H, 4.05; N, 24.01.IR (ν, $cm^{-1}$); 3515 (broad O–H), 3010 (C–H) aromatic, 2991 (C–H) aliphatic, 1713 (C=O), 1227 (Ph–O). $^1$H NMR (400 MHz; $d_6$-DMSO, δppm); 2.43 (s 3H, $CH_3$), 3.46 (s, 2H, $CH_2$), 6.84-7.62 (m, 4H, ArH), 15.42 (s, 1H, OH, disappeared by $D_2O$), $^{13}$CNMR : 14.11 ($CH_3$), 37.62 ($CH_2$), 57.58 (C-triazole), 116.95, 118.23, 125.44, 128.22, 134.45, 129.95 (ArC), 157.75 (C=N), 186.94 (C=O).

*2-(2-(2-Hydroxyphenyl)-2-methyl-5-oxo-5,6-dihydro-1H-imidazo[1,2-b][1,2,4]triazol-3(2H)-yl)propanoic acid* ***3b***. Anal. calcd. for $C_{14}H_{16}N_4O_4$ (Mr = 304.30): C, 55.20; H, 5.26; N, 18.40. Found: C, 54.87; H, 5.10; N, 18.02.IR (ν, $cm^{-1}$); 3408 (broad O–H), 3321 (O–H), 3136 (NH), 3071 (C–H) aromatic, 2981 (C–H) aliphatic, 1714 (C=O), 1617 (COOasy), 1587 (COOsy), 1236 (Ph–O). $^1$H NMR (400 MHz; $d_6$-DMSO, δppm); 2.55 (S, 3H, $CH_3$), 3.86 (d, 3H, $CH_3$), 4.09 (s, 1H, CH-COOH), 4.10 (s, 2H, $CH_2$), 5.14 (s, 2H, OH, NH, disappeared by $D_2O$), 6.91-7.66 (m, 4H, ArH), 14.40 (s, 1H, OH, disappeared by $D_2O$). $^{13}$CNMR: 14.81 ($CH_3$), 30.72 ($CH_3$), 33.53 ($CH_2$), 34.01(CH), 117.47, 118.86, 126.22, 126.86, 128.87, 132.20 (ArC), 163.68 (C=N), 169.27 (C=O), 174.13 (C=O).MS (m/$z^+$, %): 305 ($M^{+1}$, 2.95); 247 (4.81); 189.10 (6.39); 159.10 (3.70); 117.15 (22.48), 59 (100).

*2-(2-(2-Hydroxyphenyl)-2-methyl-5-oxo-5,6-dihydro-1H-imidazo[1,2-b][1,2,4]triazol-3(2H)-yl)-3-(1H-indol-2-yl)propanoic acid* ***3d***. Anal. calcd. for $C_{22}H_{21}N_5O_4$ (Mr = 419.43): C, 62.94; H, 5.06; N, 16.68. Found: C, 62.65; H, 5.10; N, 16.42.IR (ν, $cm^{-1}$); 3408 (broad O–H), 3321 (O–H),

3202, 3136 (2NH), 3050 (C–H) aromatic, 2976 (C–H) aliphatic, 1710 (C=O), 1616 (COOasy), 1586 (COOsy), 1233 (Ph–O). $^1$H NMR (400 MHz; $d_6$-DMSO, δppm); 2.55 (S, 3H, $CH_3$), 3.86 (d, 2H, $CH_2$), 4.09 (s, 1H, CH-COOH), 4.1 (s, 2H, $CH_2$), 5.14 (s, 2H, OH, NH, disappeared by $D_2O$), 6.91-7.66 (m, 8H, ArH), 14.24 (s, 1H, OH, disappeared by $D_2O$). $^{13}$CNMR: 24.81 ($CH_3$), 30.22 ($CH_2$), 33.33 ($CH_2$), 54.01 (CH), 117.47, 118.86, 122.23, 123.11, 125.76, 126.22, 126.86, 128.87, 132.20, 133.44. 136.76, 138.22 (ArC), 153.66 (C=N), 168.22 (C=O), 174.10 (C=O).

*General procedure for synthesis of compounds **4b,d***

To a solution of 2-amino-5(2-hydroxy phenyl)-1,2,4-triazole carboxylic acid **1a,d** (0.01 mol) and 4-chlorobenzoyl chloride (1.28 mL, 0.01 mol) in chloroform and a catalytic amount of triethylamine as a catalyst. The reaction component was refluxed for 7 hrs to complete the reaction (monitored by TLC). After completion of the reaction, mixture was cooled to room temperature and the solid mass of the compounds was filtered off and recrystallized from ethanol.

*2-(3-(4-Chlorobenzamido)-5-(2-hydroxyphenyl)-5-methyl-1H-1,2,4-triazol-4(5H)-yl)propanoic acid **4b***. Anal. calcd. for $C_{18}H_{17}ClN_4O_4$ (Mr = 388.80): C, 55.60; H, 4.41; N, 14.41; Cl, 9.12. Found: C, 56.70; H, 4.32; N, 14.25 ; Cl, 9.08.IR (ν, $cm^{-1}$); 3412 (broad O–H), 3268 (NH), 3171 (NH), 3071 (C–H) aromatic, 2991 (C–H) aliphatic, 1732 (C=O), 1612 (COOasy), 1589 (C=N), 1513 (COOsy), 1255 (Ph–O). $^1$H NMR (400 MHz; $d_6$-DMSO, δppm); 2.22 (d, 3H, $CH_3$), 2.58 (s, 3H, $CH_3$), 3.37 (m, 1H, CH-COOH), 7.35-8.23 (m, 8H, 2ArH), 8.23 (s, 1H, OH, exchangeable by $D_2O$), 9.17 (s, 1H, NH, disappeared by $D_2O$), 10.21 (s, 1H, OH, disappeared by $D_2O$). $^{13}$CNMR: 18.04 ($CH_3$), 37.86 ($CH_3$), 61.94 (C-triazole), 118.45, 122.23, 123.84, 126.55, 128.45, 132.50. 134.67, 141.20, 143.44, 144.29 (ArC), 152.35 (CH=N), 164.33 (C-OH), 179.47 (C=O), 198.56 (C=O).

*2-(3-(4-Chlorobenzamido)-5-(2-hydroxyphenyl)-5-methyl-1H-1,2,4-triazol-4(5H)-yl)-3-(1H-indol-2-yl)propanoic acid **4d***. Anal. calcd. for $C_{27}H_{24}ClN_5O_4$ (Mr = 517.96): C, 62.61; H, 4.67; N, 13.52; Cl, 6.84. Found: C, 62.45; H, 4.55; N, 13.35 ; Cl, 6.62. IR (ν, cm-1); 3437 (broad O–H), 3315 (OH), 3162, 3111 (2NH), 3018 (C–H) aromatic, 2987 (C–H) aliphatic, 1703 (C=O), 1606 (COOasy), 1587 (C=N), 1536 (COOsy), 1227 (Ph–O). $^1$H NMR (400 MHz; $d_6$-DMSO, δppm); 2.24 (s, 3H, $CH_3$), 3.25, 3.49 (dd, 2H, $CH_2$), 3.62 (t, 1H, CH-COOH), 4.49 (s, 1H, CH-indole), 6.98-8.13 (m, 12H, 3ArH), 8.65 (s, 1H, NH, disappeared by $D_2O$), 10.11 (s, 1H, OH, exchangeable by $D_2O$), 10.87 (s, 1H, NH, disappeared by $D_2O$), 10.90 (s, 1H, NH, disappeared by $D_2O$), 12.91(s, 1H, OH, exchangeable by $D_2O$). $^{13}$CNMR: 19.39 ($CH_3$), 26.23 ($CH_2$), 56.24 (CH-COOH), 61.94 (C-triazole), 111.79 (CH-indole), 117.02, 118.22, 122,34, 123.44, 124.43, 126.32, 126.88, 128.22, 1,32.11, 133.45, 134,67, 136.74, 138.55, 141.22, 142.21, 143.11 (ArC), 156.91 (C=N), 164.89 (C-OH), 173.78 (C=O), 180.56 (C=O). MS ($m/z^+$, %): 515.20 ($M^{-2}$, 0.04); 481 (0.05), 437 (0.17), 317 (1.78); 201 (5.20); 175.10 (3.70); 159.15 (5.62), 130.15 (24.08); 103.10 (10.07); 59 (100).

*General procedure for synthesis of compounds **5a, 6a***

*Method A*. 2-Amino-5(2-hydroxy phenyl)-1,2,4-triazole carboxylic acid **1a** (0.01 mol) was dissolved in 3 mL thionyl chloride and kept for 24 hrs at room temperature. After completion of the reaction, the mixture was poured into petroleum ether drop-wise and the solid mass was filtered off and recrystallized from ethanol into compound **5a**.

*Method B*. An equimolar mixture of 2-amino-5(2-hydroxy phenyl)-1,2,4-triazole carboxylic acid **1a** (0.01 mol) and thionyl chloride (1.2 g, 0.01 mol) was dissolved in 3 mL DMF. The reaction mixture was refluxed for 3 hrs (as monitored by TLC). After completion of the reaction, mixture

was poured into ice and the solid mass was filtered off and recrystallized from ethanol into compound **6a**.

*2-(3-Amino-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-4(5H)-yl)propanoyl chloride* ***5a***. Anal. calcd. for $C_{11}H_{13}Cl\ N_4O_2$ (Mr = 268.70): C, 49.17; H, 4.88; N, 20.85; Cl, 13.19. Found: C, 49.77; H, 4.78; N, 20.51; Cl, 13.05. IR (ν, $cm^{-1}$); 3353 (OH), 3224, 3136, 3024 (NH, $NH_2$), 3010 (C–H) aromatic, 2929 (C–H) aliphatic, 1653 (C=O), 1584 (C=N), 1269 (Ph–O). $^1$H NMR (400 MHz; $d_6$-DMSO, δppm); 1.04 (d, H, $CH_3$), 3.47 (s, 1H, CH-triazole), 3.65 (q, 1H, CH-COOH), 6.17 (s broad, 3H, $NH_2$, OH, exchangeable by $D_2O$), 7.42-8.07 (m, 4H, ArH) 8.82 (s, 1H, NH, exchangeable by $D_2O$). $^{13}$CNMR: 18.04 ($CH_3$), 57.35 (CH), 62.31 (CH-triazole), 118.67, 122.89, 123.55, 126.76. 130.84, 133.29 (ArC), 154.23 (C=N), 167.47(C=O).

*3-(2-Hydroxyphenyl)-5-methyl-5,7-dihydro-2H-imidazo[2,1-c][1,2,4]triazol-6(3H)-one* ***6a***. Anal. calcd. for $C_{11}H_{12}N_4O_2$ (Mr = 232.24): C, 56.89; H, 5.21; N, 24.12. Found: C, 56.35; H, 5.65; N, 23.99. IR (ν, cm-$^1$); 3370 (OH), 3243, 3179 (2NH), 3040 (C–H) aromatic, 2970 (C–H) aliphatic, 1661 (C=O), 1584 (C=N), 1269 (Ph–O). $^1$H NMR (400 MHz; $d_6$-DMSO, δ ppm); 1.06 (d, H, $CH_3$), 3.46 (s, 1H, CH-triazole), 5.02 (q, 1H, CH-imidazole), 6.96-7.11 (m, 4H, ArH), 10.27, 10.71, 11.13 (s, 3H, 2NH, OH, exchangeable by $D_2O$ ).

*General method for preparation of* ***7a, c, d, e***

An equimolar of 2-amino-5(2-hydroxy phenyl)-1,2,4-triazole carboxylic acid **1a, c, d , e** (0.01 mol) and 5 mL Acetic anhydride in presence of few drops of pyridine. The reaction was refluxed for 2-4 hrs (monitored by TLC). After completion of the reaction, the mixture was poured into ice and the solid mass of the compounds was filtered and recrystallized from ethanol.

*2-(3-acetamido-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-4(5H)-yl)propanoic acid* ***7a***. Anal. calcd. for $C_{13}H_{16}N_4O_3$ (Mr = 292.29): C, 53.37; H, 5.47; N, 19.16. Found: C, 52.97; H, 5.16; N, 18.93. IR (m, cm-1); 3218 (O-H), 3224, 3149 (2NH), 3067 (C–H) aromatic, 2930, 2870 (C–H) aliphatic, 1689 (C=O), 1663 (C=N), 1612 (COOasy), 1486 (COOsy), 1179 (C-O), 1280 (Ph–O). $^1$H NMR (400 MHz; $d_6$-DMSO, ppm); 2.05 (d, 3H, $CH_3$), 2.22 (s, 3H, $COCH_3$), 2.55 (t, 1H, CH-COOH), 4.32 (s, 1H, CH-triazole), 7.05-7.32 (m, 4H, ArH), 7.94 (s, 1H, NH, disappeared by $D_2O$), 11.60 (s, 1H, NH, disappeared by $D_2O$), 11.97 (s, 1H, OH, exchangeable by $D_2O$), 14.22 (s, 1H, OH, exchangeable by $D_2O$). $^{13}$CNMR; 21.23 ($CH_3$), 22.87 ($COCH_3$), 43.26 (CH-COOH), 53.52 (CH-triazole), 118.78, 122.35, 124.89, 126.66, 132.34, 134.29 (ArC), 148.33 (C=N), 168.67(C=O), 172.28 (C=O). MS (m/z$^+$, %): 292.20 ($M^{+1}$, 5.95); 291 (6.39); 278 (26.09); 247 (3.93); 236 (44.03); 194.10 (11.07); 117.10 (20.57); 59 (100).

*2-(3-Acetamido-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-4(5H)-yl)-3-(1H-indol-2-yl)propanoic acid* ***7c***. Anal. calcd. for $C_{21}H_{21}N_5O_4$ (Mr = 407.42): C, 61.88; H, 5.15; N, 17.81. Found: C, 61.54; H, 5.20; N, 17.55. IR (KBr, ν, $cm^{-1}$); 3303 (O–H), 3230, 3188 (2NH), 3142 (NH indole), 3059 (C–H) aromatic, 2927 (C–H) aliphatic, 1695 (C=O), 1641 (C=N), 1620 (COOasy), 1487 (COOsy), 1188 (C–O). $^1$H NMR (400 MHz; d6-DMSO, δ ppm): 1.92 (s, 3H, $COCH_3$), 2.01, 2.19 (dd, 2H, $CH_2$), 2.25-2.33 (t, 1H, CH-COOH), 5.19 (s, 1H, CH-triazole), 6.82 (s, 1H, CH-indole), 6.97-7.54 (m, 8H, 2ArH), 8.88 (s, 1H, NH, exchangeable by $D_2O$), 9.66 s, 1H, OH, disappeared by $D_2O$), 11.81 (s, 1H, OH, exchangeable by $D_2O$), 11.99 (s, 1H, NH, disappeared by $D_2O$). $^{13}$CNMR: 22.21 ($COCH_3$), 26.83 ($CH_2$), 39.55 (CH-COOH), 53.48 (CH-triazole), 112.22 (CH-indole), 118.86, 122.55, 123.44, 124.79, 132.33, 133.74 (ArC), 149.24 (C=N), 168.22 (C=O), 172.72 (C=O).

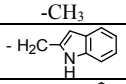
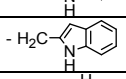
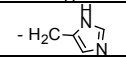
*2-(3-Acetamido-5-(2-hydroxyphenyl)-5-methyl-1H-1,2,4-triazol-4(5H)-yl)-3-(1H-indol-2-yl)propanoic acid* ***7d***. Anal. calcd. for $C_{22}H_{23}N_5O_4$ (Mr = 421.45): C, 62.64; H, 5.45; N, 16.60. Found: C, 62.44; H, 5.20; N, 16.95. IR (KBr, ν, $cm^{-1}$); 3461 (O–H), 3311, 3180 (2NH), 3142 (NH-indole), 3066 (C–H) aromatic, 2989 (C–H) aliphatic, 1698 (C=O), 1632 (C=N), 1607 (COOasy), 1486 (COOsy), 1173 (C–O). $^1$HNMR (400 MHz; $d_6$-DMSO, δ ppm): 1.92 (s, 3H, $COCH_3$), 2.06, 2.20 (dd, 2H, $CH_2$), 2.30 (s, 3H, $CH_3$), 2.85 (t, 1H, CH-COOH), 6.89 (s, 1H, CH-indole), 6.98-7.58 (m, 8H, 2ArH), 7.61 (s, 1H, NH, exchangeable by $D_2O$), 8.18 (s, 1H, NH, exchangeable by $D_2O$), 11.81 (s, 1H, OH, exchangeable by $D_2O$), 11.97 (s, 1H, NH, disappeared by $D_2O$). $^{13}$CNMR: 21.43 ($CH_3$), 22.24 ($COCH_3$), 25.23 ($CH_2$), 43.26 (CH-COOH), 62.65 (C-triazole), 123.22 (CH-indole), 118.66, 121.32, 122.50, 123.79, 124.62, 126.55, 127.32, 128.45, 132.98, 132.61, 133.74 (ArC), 148.70 (C=N), 168.03 (C=O), 172.28 (C=O).

*2-(3-Acetamido-5-(2-hydroxyphenyl)-1H-1,2,4-triazol-4(5H)-yl)-3-(1H-imidazol-5-yl)propanoic acid* ***7e***. Anal. calcd. for $C_{16}H_{18}N_6O_4$ (Mr = 358.35): C, 53.58; H, 5.02; N, 23.44. Found: C, 53.33; H, 5.21; N, 23.22. IR (KBr, ν, $cm^{-1}$); 3362 (O–H), 3234, 3187 (2NH), 3132 (NH-imidazole), 3061 (C–H) aromatic, 2968 (C–H) aliphatic, 1687 (C=O), 1612 (C=N), 1561 (COOasy), 1446 (COOsy), 1186 (C–O). $^1$HNMR (400 MHz; $d_6$-DMSO, δ ppm): 1.87 (s, 3H, $COCH_3$), 2.05, 2.26 (dd, 2H, $CH_2$), 3.44 (t, 1H, CH-COOH), 4.24 (s, 1H, CH-triazole), 7.10-7.81 (m, 6H, ArH + 2CH-imidazole),7.68 (s, 1H, OH, disappeared by $D_2O$), 8.27 (s, 1H, NH, exchangeable by $D_2O$), 11.56 (s, 1H, NH indole, disappeared by $D_2O$), 11.77 (s, 1H, OH, disappeared by $D_2O$). $^{13}$CNMR: (400 MHz; $d_6$-DMSO, δ ppm): 21.24 ($COCH_3$), 28.53 ($CH_2$), 56.52 (CH-COOH), 76.05 (CH-triazole), 123.85, 134.22 (2CH-imidazole), 118.80, 122.23, 126.86, 132.85, 133.35 (ArC), 153.90 (C=N), 168.69, 169.78 (CO), 181.82 (C=O).

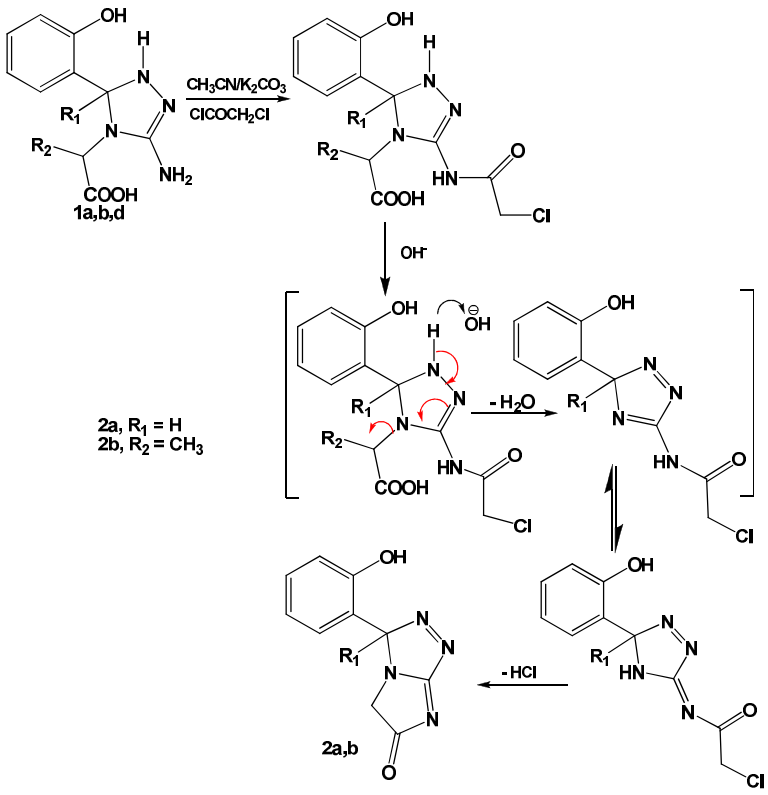
## RESULT AND DISCUSSION

The starting material amino acid coupled triazoles **1a-e** [29] were allowed to react with different reagents such as acid chloride derivatives, thionyl chloride acetic anhydride then a comparative study of the antileishmanial activity was achieved on the synthesized compounds searching for structure-activity relationship information to support the development of new drug candidates for *Leishmanial aethiopica promastigotes* (Table 1).

Table 1. Characterization data of amino acid coupled triazole derivatives (**1a-e**).

| Compound No. | $R_1$ | $R_2$ | M.P. (°C) | Yield (%) |
|---|---|---|---|---|
| **1a** | H | $-CH_3$ | 267 | 52 |
| **1b** | $CH_3$ | $-CH_3$ | 212 | 61 |
| **1c** | H |  | 291 | 57 |
| **1d** | $CH_3$ |  | 257 | 59 |
| **1e** | H |  | 272 | 48 |

The reaction of compounds **1a,b** and **1d** with chloroacetyl chloride in acetonitrile/water as a solvent and in the presence of potassium carbonate as catalyst (method A) afforded the unexpected products imidazo-1,2,4-triazole **2a,b**. The reaction mechanism of formation of the unexpected products **2a,b** was suggested to proceed *via* acetylation of amino group in first step then a preliminary elimination of an acid molecule followed by tautomerization and cyclization, Scheme 1.

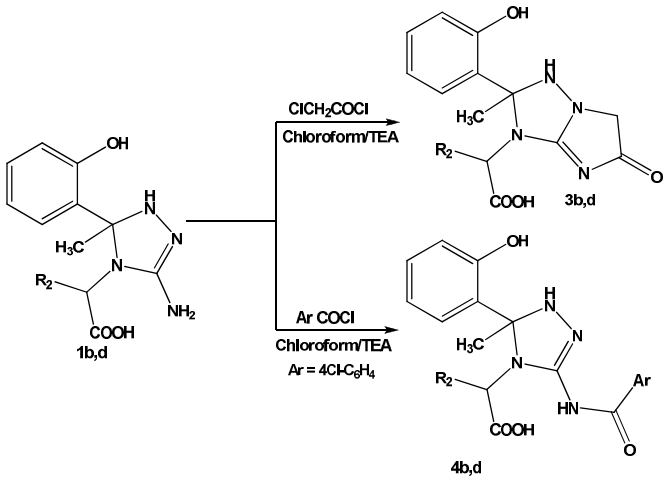



Scheme 1

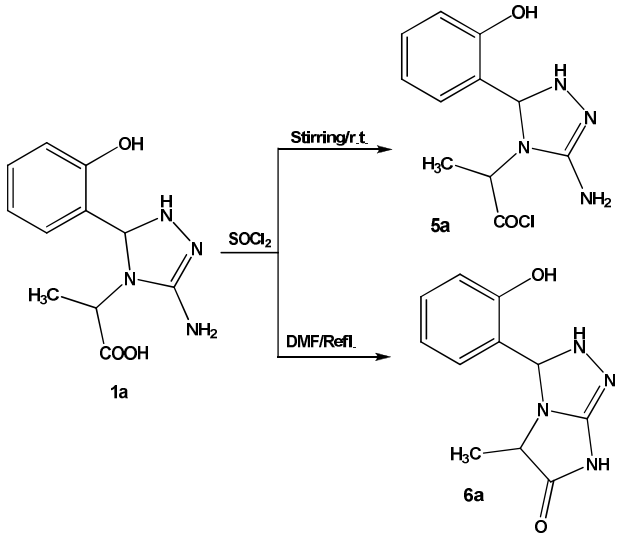
The structures of the synthesized compounds were assigned based on spectral data (IR, $^{1}$H-NMR, $^{13}$C-NMR, MS) and elemental analysis. IR spectra of compounds **2a,b** showed new peak owing to the new carbonyl group of imidazole ring located at 1695 and 1713 cm$^{-1}$, respectively. $^{1}$H-NMR spectra showed the presence of protons of $R_1$ where noted singlet signal at 3.99 ppm for $CH_{triazole}$ group in case of compound **2a**, also singlet signal at 2.43 ppm for $CH_3$ group in case of compound **2b**. Moreover, showed disappearance of protons of $R_2$, CH and COOH groups which noted in starting compound, in addition to the existence of new singlet signal of $CH_2$ group of imidazole ring at 3.92 and 4.02 ppm corresponding to compound **2a** and **2b**, respectively. $^{13}$C-NMR spectrum showed the disappearance of signal of carbonyl group of carboxylic acid group and appearance of new carbonyl group of imidazole ring at 170.10 and 186.94 ppm, respectively.

Reaction of compound **1b** with chloroacetyl chloride in chloroform in presence of a catalytic amount of triethylamine (method B) afforded the corresponding 5-oxoimidazo-1,2,4-triazole-propanoic acid **3b** (Scheme 2). Where IR spectrum of compound **3b** illustrated the appearance of new peaks at 1714 cm$^{-1}$corresponding to the carbonyl group, $^{1}$H-NMR showed the presence of two $CH_3$ groups and $CH_2$ group at 2.55, 3.86 and 4.01 ppm and $^{13}$C-NMR spectrum showed the appearance of two signals of two carbonyl groups at 169.27 and 174.13 ppm. Also, the reaction of compound **1b** and **1d** with 4-chlorobenzoyl chloride in chloroform in the presence of

few drops of triethylamine gave the corresponding *N*-aryl derivatives **4b** and **4d**. The IR spectra showed the presence of new peak of carbonyl group of carbonyl acid. $^{1}$H-NMR illustrated increase the protons number due to the phenyl group of chlorobenzoyl chloride. $^{13}$C-NMR spectrum of compound **4b** showed appearance of two signals of two carbonyl groups at 179.47 and 198.56 ppmand 173.79 and 180.56 ppm for compound **4d**, Scheme 2.




Scheme 2

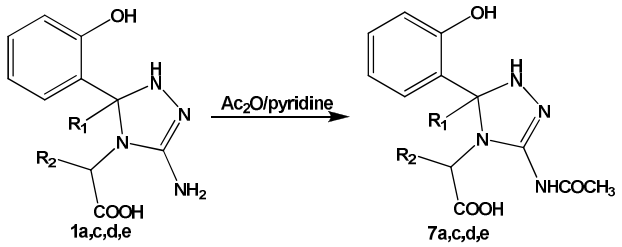



Scheme 3

Moreover, compound **1a** could react with thionyl chloride in different conditions: at room temperature afforded the corresponding 2-[3-Amino-5-(2-hydroxy-phenyl)-1,5-dihydro-[1,2,4]triazol-4-yl]-propionyl chloride **5a**. Under reflex, the reaction of the titled compound **1a** with thionyl chloride in dimethylformamide gave 2,3-dihydro-3-(2-hydroxyphenyl)-5-methyl-

5H-imidazo[2,1-c][1,2,4]triazol-6(7H)-one **6a**, Scheme 3. As observed from IR spectra the disappearance of COOH group and appearance of C=O group at 1637 $cm^{-1}$ in case of compound **5a** and at 1701 $cm^{-1}$ in case of compound **6a**. $^{1}$H-NMR and $^{13}$C-NMR spectra confirmed the suggested structure of the obtained compounds, Scheme 3.

Acetylation reaction of 1,2,4-triazole-3-carboxylic acid derivatives **1a, c,d,g** with acetic anhydride in presence of few drops of pyridine gave *N*-acetylacetamido-1,2,4-triazol-4(*5H*)-propanoic acid **7a,c,d,e** Scheme 4. IR spectrum illustrated the appearances of new carbonyl group owing to acetyl group. $^{1}$H-NMR spectrum of compounds **7a,c,d,e** showed singlet signals of acetyl group at 2.22, 1.92, 1.87 and 1.92 ppm, respectively. $^{13}$C-NMR of these compounds observed two signals of carbonyl group, one of carboxylic acid and the other for acetyl groups which proven the expected chemical structure.




Scheme 4

*In vitro anti leishmanial activity on Leishmanial aethiopica promastigotes*

The Alamar blue indicator (oxidation-reduction®) was enhanced to mensuration cytotoxicity of new heterocyclic compounds against the protozoan parasite L.aethiopica by using a quantitative colorimetric method. Alamar blue assay was used to measure the viability of promastigotes and determine the ant leishmanial activity of the synthesized compounds [30-34] (supporting information). To evaluate the ant leishmanial activity of the synthesized compounds, a final concentration of 1 mg/mL of synthesized compounds dissolvedin DMSO. There are no effects on parasite when the final concentration of DMSO did not exceed 0.1%. Test and standard solutions were diluted to suitable concentrations using fresh complete media. The test compounds were prepared by three-fold serial dilutions (starting from 10 to 0.04 mg/mL)

The test compounds were used in three-fold serial dilutions to evaluate its antileishmanial activity according to Amphotericin B deoxycholate and miltefosine were used as positive controls for comparison. Promastigote type of *L. aethiopica* was used for the assay. A 100 mL of culture media containing $3x10^{6}$ promastigotes of *L. aethiopica* were seeded in each well of a 96 well flat bottom plate. Different dilutions of test compounds (10, 3.33, 1.11, 0.37, 0.12, 0.04 mg/mL) were added to the parasites. The assay was done in duplicates. The parasites existed only in wells; media and DMSO were used as negative control. The plates were remained at room temperature (21±1 $^{o}$C). After 24 h, 10 mL wasadded to each of the wells of Alamar blue (12.5 mg resazurin dissolved in 100 mL of distilled water) [32]. After 48 h, absorbance of the resulting mixture was measured at 540 and 630 nm using a plate reader. Alamar blue works through the conversion of resazurin (7-hydroxy-*3H*-phenoxazine-3-one-10-oxide), the active ingredient of Alamar blue® (blue and non-fluorescent), to resorufin (pink and highly fluorescent) through reduction reactions of metabolically active cells. There is a proportional relationship between the amount of fluorescence produced and the number of living cells.

All the synthesized compounds had $IC_{50}$ better than standard drugs miltefosine and comparative activity to amphotericin B deoxycholate. The lead compound **7d** showed more activity than amphotericin B and about 110 folds than miltefosine. Moreover, compounds **2b** and **7e** showed similar activity to amphotericin Band high activity to miltefosine, which

indicates its ant leishmanial activity against L. aethiopica. In deeps, compounds **6a, 7a, 7c** showed low activity corresponding to amphotericin B and more patent than miltefosine against L. aethiopica promastigotes (Table 2). The bar chart (Figure 1) illustrated the resulted data of the tested compound comparing to reference drugs.

Table 2. Antipromastigote activity ($IC_{50}$) of the synthesized compounds.

| Compound | $IC_{50}$ values (µg/mL) |
|---|---|
| 2a | 2.1144±0.24 |
| 2b | 0.0666±0.02 |
| 3b | 1.0836±0.22 |
| 4a | 2.0986±0.21 |
| 5a | 1.0986±0.21 |
| 6a | 0.6247±0.28 |
| 7a | 0.3016±0.14 |
| 7c | 0.2671±0.26 |
| 7d | 0.0307±0.11 |
| 7e | 0.0876±0.22 |
| Miltefosine | 3.1924±0.14 |
| Amphotericin B deoxycholate | 0.0472±0.02 |

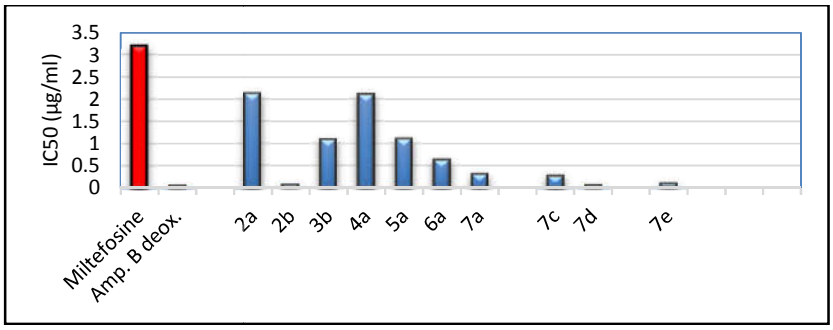



Figure 1. The test compounds showed the highest antileishmanial activity, compared with the reference.

*In vivo acute toxicity testing*

The most active ant leishmanial compounds, **2b, 7d** and **7e** were tested for their toxicity in mice (supporting information) [35, 36]. The experimental mice did not have any toxicity signs after treatment with the test compounds. There was no significant difference in the weight of the mice and no death cases were recorded during 3 days of observation post administration of the test compounds (Table 2). The test compounds were well tolerated by the experimental animals orally up to 250 mg/kg. Eleven groups of mice, each group consisting of six male mice (25-30 g) were used for testing acute toxicity [30]. The mice in each group were fasted overnight and weighed prior to test. The compounds were prepared in suspension form in aqueous vehicle containing 1% gum acacia. Mice in group one to ten were given 25, 50, 100, 200 and 350 mg/kg of the synthesized compounds as a single dose for only one day, while the eleventh group was treated orally with the vehicle gum acacia (control group) at a maximum dose of 1 mL/100 g of body weight. The mortality percentage in each group was recorded after 24 h. Additionally, the test compounds were investigated for their parenteral acute toxicity in groups of six mice each as reported earlier. The compounds, or their vehicle, propylene glycol (control), were given by intraperitoneal injection in doses of 10, 25, 50, 75, 100 mg/kg. The survival percentage was followed up to seven days.

*Molecular docking*

In the present study, molecular docking was performed to rationalize the obtained biological results. The interactions of the synthesized compounds with the active site of target macromolecules were investigated to study the mode of binding and their orientations and related to their ant leishmanial activity. The binding site of *Leishmania* major pteridine reductase LmPTR1 (PDB ID: 2BFM) was explored computationally, were carried out using Molecular Operating Environment (MOE Dock 2016) software [37]. The crystal structure of LmPTR1 with the bound TOP (PDB ID: 2BFM) was downloaded from the protein data bank. PTR1 represents a target for the development of improved therapies for infections caused by this protozoan. Target compounds were constructed using the builder interface of the MOE program and all hydrogens were added. Conformational analyses were done through energy minimization using Force Field MMFF94x. The active sites of both proteins were generated using the MOE-Alpha Site Finder, and then ligands were docked within this active site using MOE Dock.

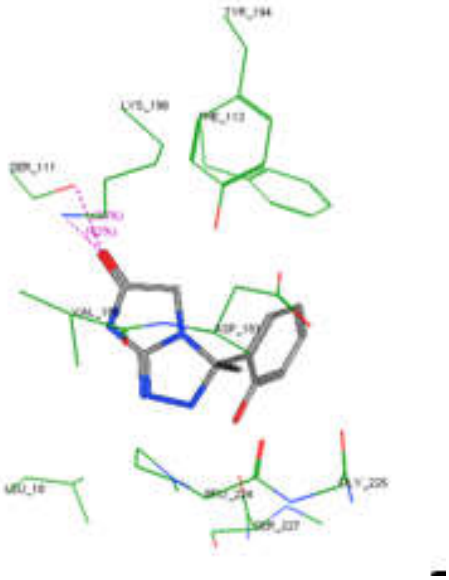


Figure 2. Docking of compound **2b**.

For example, the determination of three dimensional cocrystal structure of LmPTR1 complex with 3-(2-hydroxyphenyl)-3-methyl-3H-imidazo[2,1-c][1,2,4]triazol-6(5H)-one **2b** (trimethoprim, TOP) showed hydrogen bond interaction with Ser 111 as well as hydrophobic interactions with Lys 198 and other amino acid residues (Figure 2).

## CONCLUSION

The objectives of the present study were to synthesize, characterized and investigate the ant leishmanial activities of some imidazo-1,2,4-triazole moiety serving as more potent dual ant leishmanial agents. The *in vitro* anti promastigote activity showed that $IC_{50}$ value of compounds **2b, 6a, 7a, 7c** and **7e** better than standard drugs miltefosine and comparative activity to amphotericin B deoxycholate. The superior compound **7d** showed more activity than amphotericin B and about 110 folds more active than miltefosine. These findings were supported by the docking for **2b** compound, which demonstrated that this compound established hydrogen bonding with some amino acid residues in LmPTR1 active site, which showed good

binding profile in addition to some hydrophobic interactions with good scoring results. Toxicity studies for the most active compounds indicated their safety orally and parenterally up to 300 and 100 mg/kg, respectively. In conclusion, compounds **2b, 7d** and **7e** demonstrated dual activity against leishmania and represent fruitful scaffolds for the development of dual acting ant leishmanial agents.

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