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Comparative studies of novel triazole derivative having chiral center and their antimicrobial activities

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ABSTRACT

(-)5-[(1S)amino (2-chlorophenyl)methyl]-4-substituted phenyl-1,2,4-triazole-3-thiol ***(11a-j)*** *& (+)5- [(1S)amino (2-chlorophenyl) methyl]-4-substituted phenyl-1,2,4-triazole-3-thiol* ***(12a-j)*** *have been synthesized by the reaction of (-)5-[(1S)amino(2-chlorophenyl)methyl]-1,3,4-oxadiazole-2-thiol* ***(9)*** *& (+)5-[(1S)amino(2-chlorophenyl)methyl]-1,3,4-oxadiazole-2-thiol* ***(10)*** *with con. H_2SO_4, L(+) Tartaric acid, L(-) Tartaric acid, Hydrazine Hydrate, carbon disulfide and Various aromatic amine respectively. These compounds have been screened for their antibacterial and antifungal activities against different microorganisms. The structures of novel synthesized compounds have been established on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.*

Keywords: 1,2,4-Triazole, L(+) Tartaric acid, L(-) Tartaric acid, Hydrazine Hydrate.

INTRODUCTION

Triazoles have been shown to possess desirable features in medicinal chemistry. The triazoles are stable to acid and basic hydrolysis and reductive and oxidative conditions, because of their high aromatic stabilization. In addition, this heterocycle has a high dipole moment and might participate actively in hydrogen bond formation as well as in dipole–dipole and π stacking interactions[1]. Last, this compound is relatively resistant to metabolic degradation. For many years, alkylating agents have been studied with regard to cancer chemotherapy, and this has led to the development of many new and more selective alkylating agents including molecules that are based on the triazole moiety.

Various derivatives of 1,2,4-triazoles possess a wide spectrum of activity ranging from antibacterial[2,3], antifungal, anti-inflammatory[4,5], anticonvulsant, antitubercular[6], antimalarial, antiviral[7], anticancer[8], anti-TB, antiproliferative[9] and antimicrobial[10,11] activity.

MATERIALS AND METHODS

General procedure. Melting points were determined by open capillaries and are uncorrected. The homogenecity of the compounds was checked by TLC (silica gel H, BDH, Toluene: Methanol 8:2). IR-spectra (cm^{-1}) were recorded on a Shimandzu FT-IR spectrophotometer using KBr pellet method. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz & 75 MHz NMR instrument, using DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ, ppm). Mass spectra were obtained on a JMS-T100LC, Accu TOF Mass spectrometer

(DART).The elemental analysis (C,H,N) of compounds was carried out on a Carlo Erba 1108 elemental analyzer. Their results were found to be in good agreement with the calculated values.

Step 1: Synthesis of methyl amino(2-chlorophenyl)acetate (2) : A mixture of amino (2-chlorophenyl) acetic acid (0.54 mol) and Methanol was taken in a round bottom flask & to it con. H_2SO_4 (1 mol) added slowly below 35°C. After complete addition the reaction mixture was refluxed for 16 hrs till TLC OK (Mobile Phase Toluene: Methanol 8:2). Then methanol was distilled out completely & toluene & water added. After separation of two layers aq. layer taken & cooled to 10-15°C. Then dichloromethane was added and pH adjusted at 7.0 to 7.5 with liq. NH_3. From the layer separated, aq. layer was taken & washed with dichloromethane. Both organic layer were taken & washed with water. Organic layer dried with Na_2SO_4 and dichloromethane distilled out completely to obtained oily residue. This ester was directly used for the second stage without carrying for any further purification. Light yellow color liquid, Yield 85 %.

Step 2: Synthesis of methyl (-)(2*S*)-amino(2-chlorophenyl)acetate (tartrate salt) (3) : A mixture of L(-) Tartaric acid(0.36 mol) and methanol was taken in a round bottom flask. Reaction mixture was cooled at 15-20°C and methyl amino (2-chlorophenyl)acetate (0.50 mol) and acetone were added into the reaction mass at 15-20°C for 2 hrs. After addition temperature raised to 30-35°C which was maintained for 18 hrs. The reaction mixture was cooled at 0-5°C and maintained for 2 hrs. Then it was filtered and washed with methanol. White color product, Yield 130 %. $[\alpha]_D^{20}$ - 85° to - 95° (c=1 in methanol), Melting Range 158 °C to 163 °C.

Step 3: Synthesis of methyl (+)(2*S*)-amino(2-chlorophenyl)acetate (tartrate salt) (4) : A mixture of L(+) Tartaric acid(0.36 mol) and methanol was taken in a round bottom flask. Reaction mixture was cooled at 15-20°C; then methyl amino(2-chlorophenyl)acetate (0.50 mol) and acetone were added into the reaction mass at 15-20°C for 2 hrs. After addition temperature raised to 30-35°C which was maintained for 18 hrs. The reaction mixture were cooled to 0-5°C and maintained for 2 hrs. It was then filtered & washed with methanol. White color product, Yield 130 %. $[\alpha]_D^{20}$ + 85° to + 95° (c=1 in methanol), Melting Range 164 °C to 168 °C.

Step 4: Synthesis of Free Base from tartrate salt (5) : A mixture of methyl (-)(2*S*)-amino(2-chlorophenyl)acetate tartrate salt, water and dichloromethane were taken into a round bottom flask pH was adjusted to 7.0 - 8.0 with liq. NH_3. From the layer separated, aqueous layer was taken & washed with dichloromethane. Both Organic layer were taken and dry with Na_2SO_4 and dichloromethane distilled out completely to obtained oily residue. $[\alpha]_D^{20}$ - 130° to - 135° (c = 1 in methanol), Yield 52%.

Step 5: Synthesis of Free Base from tartrate salt (6) : A mixture of methyl (+)(2*S*)-amino(2-chlorophenyl)acetate tartrate salt, water and dichloromethane were taken into a round bottom flask. pH was adjusted to 7.0 - 8.0 with liq. NH_3. From the layer separated, aqueous layer was taken & washed with dichloromethane. Both Organic layer were taken and dry with Na_2SO_4 and dichloromethane distilled out completely to obtained oily residue. $[\alpha]_D^{20}$ + 130° to +135° (c = 1 in methanol), Yield 52%.

Step 6: Synthesis of (-) (2*S*)-2-amino-2-(2-chlorophenyl) acetohydrazide (7) : A mixture of (-) (2*S*)-amino (2-chlorophenyl) acetate (1 mol) and methanol were taken in a round bottom flask. The reaction mixture heat up to 50°C to 55°C. Hydrazine Hydrate (1.7mol) was added slowly and methanol was used as a solvents. The reaction mixture was refluxed on water bath for 5-6 hrs. The completion of the reaction was judged by TLC (Mobile Phase Toluene: Methanol 8:2). After completion of the reaction 80% of the solvent was distilled out and the reaction mixture was cooled at 5°C -10°C. The solid thus separated was collected by filtration & dried. Off white color product, Yield 75 %. $[\alpha]_D^{20}$ - 84° to - 90° (c = 1 in methanol), Melting Range 94°C - 98°C.

Step 7: Synthesis of (+)(2*S*)-2-amino-2-(2-chlorophenyl)acetohydrazide (8) : A mixture of (+) (2*S*)-amino (2-chlorophenyl) acetate (1 mol) and methanol were taken in a round bottom flask. The reaction mixture was heated up to 50°C to 55°C. Hydrazine Hydrate (1.7mol) was added slowly and methanol was used as a solvents. The reaction mixture was refluxed on water bath for 5-6 hrs. The completion of the reaction was judged by TLC (Mobile Phase Toluene: Methanol 8:2). After completion of the reaction 80% of the solvent was distilled out and the reaction mixture was cooled at 5°C -10°C. The solid thus separated was collected by filtration & dried. Off white color product, $[\alpha]_D^{20}$ + 84° to + 90° (c = 1 in methanol), Melting Range 90°C - 96°C, Yield 75 %.

Step 8: Synthesis of (-)5-[(1*S*)amino(2-chlorophenyl)methyl]-1,3,4-oxadiazole-2-thiol (9) : A mixture of (-)(2*S*)-2-amino-2-(2-chlorophenyl)acetohydrazide (1 mol) , potassium hydroxide (1 mol) and carbon disulphide (5 ml) in ethanol (50 ml) was refluxed on water bath. Temperature was maintained for 18-20 hrs at reflux till TLC OK (Mobile Phase Toluene: Methanol 8:2). After reaction was completed 50 % ethanol was distilled out. Reaction mixtures was cooled to 5°C -10°C. Filtered and dried. Light Brown color product, Melting Range 163°C - 168°C, Yield 58 %.

Step 9: Synthesis of (+)5-[(1*S*)amino(2-chlorophenyl)methyl]-1,3,4-oxadiazole-2-thiol (10) : A mixture of (+)(2*S*)-2-amino-2-(2-chlorophenyl)acetohydrazide (1 mol) , potassium hydroxide (1 mol) and carbon disulphide (5 ml) in ethanol (50 ml) was refluxed on water bath. Temperature was maintained for 18-20 hrs at reflux till TLC OK (Mobile Phase Toluene: Methanol 8:2). After reaction was completed 50 % ethanol was distilled out. Reaction mixtures was cooled to 5°C -10°C. Filtered and dried. Light Brown color product, Melting Range 109°C - 115°C, Yield 62 %.

Step 10: Synthesis of (-)5-[(1*S*)amino (2-chlorophenyl)methyl]-4-substituted phenyl-1,2,4-triazole-3-thiol (11a-j) : A mixture of (-)5-[(1S)amino(2-chlorophenyl)methyl]-1,3,4-oxadiazole-2-thiol (1 mol) & aromatic amine (1.2 mol) was refluxed in Glacial acetic acid for 6 hr. The resulting solution was then concentrated, cooled and poured into ice cold water. The solid thus separated out was filtered, dried and recrystallized from Methanol.

11a. IR (KBr νcm^{-1}): –Cl (754), -C=N (1442), -SH (2590), -NH_2 (3064). **^{1}H NMR [300 MHz, δ]:** 2.118 (s, 1H, -CH), 3.360 (s, 1H, -SH), 5.018 (s, 2H, -NH_2), 7.318-7.927 (m, 9H, ArH). **^{13}C NMR [75 MHz, δ]:** 126.3 (C_1),122.6 (C_2), 126.1 (C_3), 127.6 (C_4), 133.2 (C_5), 143.8 (C_6), 50.7 (C_7), 158.0 (C_8), 168.9 (C_9), 148.5 (C_{10}), 129.6 (C_{11}), (C_{15}), 128.7 (C_{12}), (C_{14}), (C_{13}). **M/S (m/z ,relative intensity):** 316(M^+) , 318(M+2).

11c. IR (KBr νcm^{-1}): – Cl (756), -C=N (1440), -SH (2592), -NH_2 (3060). **^{1}H NMR [300 MHz, δ]:** 2.110 (s, 1H, -CH), 3.305 (s, 1H, -SH), 4.879 (s, 2H, -NH_2), 7.266-7.553 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 122.5 (C_1),114.8 (C_2), 117.7 (C_3), 120.8 (C_4), 131.1 (C_5), 138.9 (C_6), 57.8 (C_7), 147.1 (C_8), 171.7 (C_9), 141.1 (C_{10}), 129.8 (C_{11}), (C_{15}), 127.0 (C_{12}), (C_{14}), 130.05 (C_{13}). **M/S (m/z ,relative intensity):** 352(M^+) (-1), 356(M+4).

11f. IR (KBr νcm^{-1}): – Cl (776), -OCH_3 (1091), -C=N (1408), -SH (2502), -NH_2 (3010). **^{1}H NMR [300 MHz, δ]:** 2.027 (s, 1H, -CH), 3.318 (s, 1H, -SH), 3.800 (s, 3H, -OCH_3), 5.013 (s, 2H, -NH_2), 6.893-7.703 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 126.8 (C_1), 122.4 (C_2), 125.8 (C_3), 126.9 (C_4), 134.4 (C_5), 147.4 (C_6), 53.4 (C_7), 157.4 (C_8), 177.4 (C_9), 136.4 (C_{10}), 131.8 (C_{11}), (C_{15}), 117.4 (C_{12}), (C_{14}), 160.7 (C_{13}), 55.7 (C_{16}). **M/S (m/z ,relative intensity):** 346(M^+) , 348(M+2).

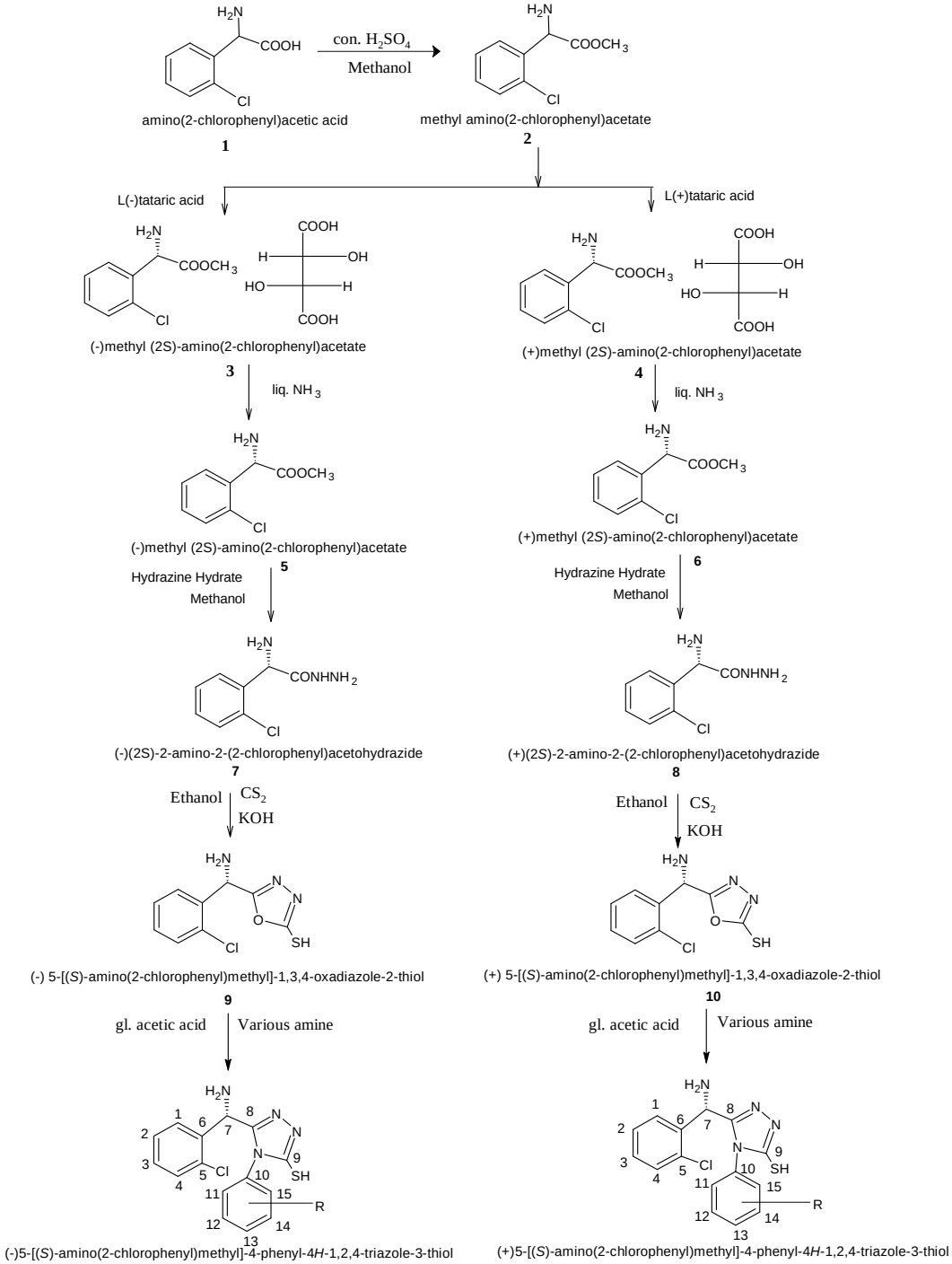
11g. IR (KBr νcm^{-1}): – Cl (781), -C=N (1461), -SH (2531), -NH_2 (3087). **^{1}H NMR [300 MHz, δ]:** 2.234 (s, 1H, -CH), 3.429 (s, 1H, -SH), 5.123 (s, 2H, -NH_2), 7.236-8.378 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 129.7 (C_1), 128.4 (C_2), 129.4 (C_3), 130.1 (C_4), 136.8 (C_5), 151.4 (C_6), 61.2 (C_7), 163.7 (C_8), 163.9 (C_9), 112.4 (C_{10}), 130.6 (C_{11}), 133.0 (C_{12}), 131.1 (C_{13}), 120.8 (C_{14}), 139.7 (C_{15}). **M/S (m/z ,relative intensity):** 360(M^+)(+1) , 362(M+2).

11h. IR (KBr νcm^{-1}): – Cl (750), -C=N (1447), -SH (2540), -CH_3 (2856), -NH_2 (3108). **^{1}H NMR [300 MHz, δ]:** 2.007 (s, 1H, -CH), 2.414 (s, 3H, -CH_3) 3.281 (s, 1H, -SH), 4.994 (s, 2H, -NH_2), 7.265-7.779 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 128.7 (C_1), 125.3 (C_2), 126.9 (C_3), 127.7 (C_4), 132.3 (C_5), 139.8 (C_6), 53.5 (C_7), 144.8 (C_8), 149.8 (C_9), 140.3 (C_{10}), 130.6 (C_{11}), (C_{15}), 129.0 (C_{12}), (C_{14}), 136.3 (C_{13}), 23.4 (C_{16}). **M/S (m/z ,relative intensity):** 332(M^+)(-2) , 334(M+2).

11i. IR (KBr νcm^{-1}): – Cl (791), -C=N (1442), -SH (2540), -CH_3 (2828), -NH_2 (3097). **^{1}H NMR [300 MHz, δ]:** 1.989 (s, 1H, -CH), 2.346 (s, 3H, -CH_3) 3.311 (s, 1H, -SH), 5.089 (s, 2H, -NH_2), 7.093-7.789 (m, 7H, ArH). **^{13}C NMR [75 MHz, δ]:** 130.1 (C_1), 126.4 (C_2), 129.7 (C_3), 129.9 (C_4), 134.7 (C_5), 143.8 (C_6), 51.1 (C_7), 153.3 (C_8), 161.5 (C_9), 126.7 (C_{10}), 119.4 (C_{11}), (C_{15}), 139.1 (C_{12}), (C_{14}), 131.2 (C_{13}), 24.6 (C_{16}),(C_{17}). **M/S (m/z ,relative intensity):** 344(M^+) , 346(M+2).

11j. IR (KBr νcm^{-1}): – Cl (771), -C=N (1455),-C=O (1691), -SH (2575), -CH_3 (2873), -NH (3021) -NH_2 (3237). **^{1}H NMR [300 MHz, δ]:** 1.986 (s, 1H, -CH), 2.109 (s, 3H, -CH_3) 3.297 (s, 1H, -SH), 5.178 (s, 2H, -NH_2), 7.208 (s, 1H, -NH), 6.987-7.825 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 125.2 (C_1), 120.3 (C_2), 126.1 (C_3), 126.8 (C_4), 131.4

(C_5), 137.8 (C_6), 50.4 (C_7), 159.7 (C_8), 160.0 (C_9), 140.5 (C_{10}), 122.2 (C_{11}), (C_{15}), 117.6 (C_{12}), (C_{14}), 138.1 (C_{13}), 173.3 (C_{16}), 21.4 (C_{17}). **M/S (m/z ,relative intensity):** 374(M^+)(-1) , 376(M+2).



where, R is
-H, 3-CH_3, 2-OH, 4-OH, 2,4-di-Cl, 2-NO_2, 4-NO_2, 4-NH_2, 3-NH_2 2-OH, 3,5 di-NH_2

Scheme I

Step 11: Synthesis of (+)5-[(1*S*)amino (2-chlorophenyl)methyl]-4-substituted phenyl-1,2,4-triazole-3-thiol (12a-j) : A mixture of (+)5-[(1*S*)amino(2-chlorophenyl)methyl]-1,3,4-oxadiazole-2-thiol (1 mol) & aromatic amine (1.2 mol) was refluxed in Glacial acetic acid for 6 hr. The resulting solution was then concentrated, cooled and poured into ice cold water. The solid thus separated out was filtered, dried and recrystallized from Methanol.

12a. **IR (KBr νcm^{-1}):** –Cl (756), -C=N (1504), -SH (2550), -NH_2 (2991). **^{1}H NMR [300 MHz, δ]:** 2.094 (s, 1H, -CH), 3.274 (s, 1H, -SH), 4.003 (s, 2H, -NH_2), 7.008-7.875 (m, 9H, ArH). **^{13}C NMR [75 MHz, δ]:** 128.4 (C_1), 125.9 (C_2), 128.9 (C_3), 129.0 (C_4), 132.8 (C_5), 142.6 (C_6), 52.8 (C_7), 147.6 (C_8), 169.7 (C_9), 142.9 (C_{10}), 130.1 (C_{11}), (C_{15}), 129.4 (C_{12}), (C_{14}), (C_{13}). **M/S (m/z ,relative intensity):** 315(M^+)(+1) , 317(M+2).

12c. IR (KBr νcm^{-1}): – Cl (810), -C=N (1510), -NH_2 (3137). **^{1}H NMR [300 MHz, δ]:** 2.500 (s, 1H, -CH), 3.400 (s, 1H, -SH), 3.860 (s, 2H, -NH_2), 6.836-7.925 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 129.8 (C_1), 126.4 (C_2), 127.4 (C_3), 137.0 (C_5), 141.98 (C_6), 56.1 (C_7), 153.3 (C_8), 171.9 (C_9), 143.0 (C_{10}), 129.3 (C_{11}), (C_{15}), 128.0 (C_{12}), (C_{14}), 132.1 (C_{13}). **M/S (m/z ,relative intensity):** 352(M^+) (-1), 356(M+4).

12f. IR (KBr νcm^{-1}): – Cl (758), -OCH_3 (1110), -C=N (1500), -SH (2482), -NH_2 (3017). **^{1}H NMR [300 MHz, δ]:** 2.084 (s, 1H, -CH), 3.345 (s, 1H, -SH), 3.616 (s, 3H, -OCH_3), 5.541 (s, 2H, -NH_2), 7.183-7.771 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 128.6 (C_1), 124.2 (C_2), 128.5 (C_3), 129.6 (C_4), 134.4 (C_5), 147.4 (C_6), 54.3 (C_7), 154.7 (C_8), 174.7 (C_9), 134.6(C_{10}), 138.1 (C_{11}), (C_{15}), 114.7 (C_{12}), (C_{14}), 167.0 (C_{13}), 57.5 (C_{16}). **M/S (m/z ,relative intensity):** 346(M^+) , 348(M+2).

12g. IR (KBr νcm^{-1}): – Cl (767), -C=N (1447), -SH (2423), -NH_2 (3144). **^{1}H NMR [300 MHz, δ]:** 1.885 (s, 1H, -CH), 2.981 (s, 1H, -SH), 4.339 (s, 2H, -NH_2), 7.347-8.036 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 127.9 (C_1), 124.4 (C_2), 128.0 (C_3), 131.1 (C_4), 136.0 (C_5), 154.4 (C_6), 61.1 (C_7), 163.0 (C_8), 163.2 (C_9), 114.8 (C_{10}), 136.3 (C_{11}), 133.0 (C_{12}), 131.1 (C_{13}), 124.9 (C_{14}), 137.9 (C_{15}). **M/S (m/z ,relative intensity):** 361(M^+) , 363(M+2).

12h. IR (KBr νcm^{-1}): – Cl (782), -C=N (1536), -CH_3 (2814), -NH_2 (3218). **^{1}H NMR [300 MHz, δ]:** 2.831 (s, 1H, -CH), 3.096 (s, 3H, -CH_3) 3.264 (s, 1H, -SH), 5.166 (s, 2H, -NH_2), 7.548-7.963 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 127.8 (C_1), 123.5 (C_2), 126.2 (C_3), 127.9 (C_4), 132.8 (C_5), 139.1 (C_6), 55.3 (C_7), 148.2 (C_8), 148.8 (C_9), 143.3 (C_{10}), 136.0 (C_{11}), (C_{15}), 129.0 (C_{12}), (C_{14}), 133.6 (C_{13}), 24.4 (C_{16}). **M/S (m/z ,relative intensity):** 330(M^+) , 332(M+2).

12i. IR (KBr νcm^{-1}): – Cl (767), -C=N (1488), -SH (2540), -CH_3 (2908), -NH_2 (3107). **^{1}H NMR [300 MHz, δ]:** 2.326 (s, 1H, -CH), 3.108 (s, 3H, -CH_3) 3.426 (s, 1H, -SH), 4.863 (s, 2H, -NH_2), 6.896-7.672 (m, 7H, ArH). **^{13}C NMR [75 MHz, δ]:** 131.1 (C_1), 124.6 (C_2), 127.7 (C_3), 129.9 (C_4), 137.1 (C_5), 148.8 (C_6), 51.4 (C_7), 153.5 (C_8), 161.0 (C_9), 127.0 (C_{10}), 117.9 (C_{11}), (C_{15}), 137.4 (C_{12}), (C_{14}), 132.1 (C_{13}), 24.3 (C_{16}),(C_{17}). **M/S (m/z ,relative intensity):** 344(M^+) , 346(M+2).

12j. IR (KBr νcm^{-1}): – Cl (756), -C=N (1514),-C=O (1579), -SH (2545), -NH (3172) -NH_2 (3302). **^{1}H NMR [300 MHz, δ]:** 1.799 (s, 1H, -CH), 2.829 (s, 3H, -CH_3) 3.261 (s, 1H, -SH), 4.632 (s, 2H, -NH_2), 7.063 (s, 1H, -NH), 7.139-8.105 (m, 8H, ArH). **^{13}C NMR [75 MHz, δ]:** 122.5 (C_1), 120.0 (C_2), 121.1 (C_3), 126.8 (C_4), 134.2 (C_5), 137.6 (C_6), 54.4 (C_7), 157.8 (C_8), 160.3 (C_9), 145.0 (C_{10}), 122.2 (C_{11}), (C_{15}), 116.8 (C_{12}), (C_{14}), 136.5 (C_{13}), 173.0 (C_{16}), 23.4 (C_{17}). **M/S (m/z ,relative intensity):** 374(M^+), 376(M+2).

RESULTS AND DISCUSSION

The compounds were synthesized as per **scheme I**
Amino (2-chlorophenyl) acetic acid **(1)** was converted to amino (2-chlorophenyl) methyl ester **(2)** using con.H_2SO_4 in presence of methanol at a reflux temperature. The optical isomer, (+) amino(2-chlorophenyl)methyl ester was separated from its (-) isomer using L-(+)-tartaric acid and (-) amino(2-chlorophenyl)methyl ester was separated from its (+) isomer using L-(-)-tartaric acid and the (-) tartrate salt **(3)** & (+) tartrate salt **(4)** thus obtained were converted into their free base **(5)** & **(6)** using liquor ammonia. The (-) tartrate salt& (+) tartrate salt with high enatiomeric purity were achieved by repeatedly heating and cooling the reaction mass till the required enantiomeric purity obtained. (-)(2*S*)-2-amino-2-(2-chlorophenyl)acetohydrazide **(7)** & (+) (2*S*)-2-amino-2-(2-chlorophenyl) acetohydrazide **(8)** were synthesized by reacting (-) amino(2-chlorophenyl)methyl ester & (+) amino(2-

chlorophenyl)methyl ester free base react with hydrazine hydrate. The targeted (-)5-[(1*S*)amino (2-chlorophenyl)methyl]-4-substituted phenyl-1,2,4-triazole-3-thiol **(11a-j)** & (+)5-[(1*S*)amino (2-chlorophenyl) methyl]-4-substituted phenyl-1,2,4-triazole-3-thiol **(12a-j)** were synthesize by refluxing (-)5-[(1*S*)amino(2-chlorophenyl)methyl]-1,3,4-oxadiazole-2-thiol **(9)** & (+)5-[(1*S*)amino(2-chlorophenyl)methyl]-1,3,4-oxadiazole-2-thiol **(10)** with various aromatic amine. Compounds **(10a-j)** and **(11a-j)** were prepared as a new product. The proposed structures of all the synthesized compounds are well supported by elemental analysis, IR, ^{1}H NMR & ^{13}C NMR data. Compounds **(5)** & **(6)** were insoluble in sodium bicarbonate solution indicating the involvement of acid. The ^{1}H NMR spectrum displayed signals for the presence of amino proton at δ 4.879 and δ 3.860. Aromatic protons were observed in the usual region as multiplet between δ 7.266-7.553 and δ 6.836-7.750.

Antimicrobial activity

Following common standard strains were used for screening of antibacterial and antifungal activities: ***E.Coli, P.Aeruginosa, S.Aureus, S.Pyogenus, C.Albicans, A.Niger, A.Clavatus*** The strains were procured from Institute of Microbial Technology, Chandigarh. DMSO was used as diluents / vehicle to get desired concentration of drugs to test upon standard bacterial strains.Each synthesized drug was diluted for obtaining 2000 microgram /ml concentration, as a stock solution. In primary screening 1000 microgram/ml, 500 microgram /ml, and 250 microgram /ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 200 microgram/ml, 100 microgram/ml, 50 microgram/ml, 25 microgram/ml, 12.5 microgram/ml and 6.250 microgram/ml concentrations. The highest dilution showing at least 99 % inhibition zone is taken as MIC.The result of this is much affected by the size of the inoculums. The test mixture should contain 10^{8} microorganism/ml.

Table I Antimicrobial activity of compound (11a-j) & (12a-j)

Minimum Inhibition Concentration							
comp.	**antibacterial**				**antifungal**		
	E.coli MTCC443	***P.aeruginosa MTCC1688***	***S.aureus MTCC96***	***S.pyogenus MTCC442***	***C.albicans MTCC227***	***A.niger MTCC282***	***A.clavatus MTCC1323***
11a	**100**	**100**	**100**	**100**	>1000	>1000	>1000
11b	250	**100**	250	500	>1000	>1000	>1000
11c	200	200	250	200	>1000	500	500
11d	**62.5**	**100**	500	500	500	>1000	>1000
11e	**100**	200	500	250	500	>1000	>1000
11f	200	250	500	500	1000	500	500
11g	200	250	500	500	250	>1000	>1000
11h	**100**	**100**	**125**	250	**200**	>1000	>1000
11i	250	**100**	200	**125**	250	>1000	>1000
11j	250	200	250	200	500	>1000	>1000
12a	200	**125**	200	500	500	500	>1000
12b	250	**100**	200	250	>1000	>1000	250
12c	250	**100**	**125**	200	>1000	>1000	250
12d	200	250	200	250	1000	500	1000
12e	250	250	250	200	500	1000	1000
12f	250	500	250	**100**	>1000	>1000	>1000
12g	**100**	250	**100**	**100**	1000	500	>1000
12h	**125**	200	**125**	**125**	500	250	>1000
12i	200	**62.5**	200	200	500	500	250
12j	250	250	250	**100**	>1000	500	1000
Gentamycin	0.05	1	0.25	0.5			
Ampicillin	100	--	250	100			
Chloramphenicol	50	50	50	50			
Ciprofloxacin	25	25	50	50			
Norfloxacin	10	10	10	10			
Nystatin					100	100	100
Greseofulvin					500	100	100

The Comparative activities of the newly synthesized compound **(11a-j)** and **(12a-j)** & the control antibiotic Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin, Greseofulvin on bacterial & fungal strains respectively were summarized in **Table I**. The compound **11a** exhibited the growth inhibition almost equal to the standard drug against all bacterial strains. The compound **12h & 12g** also exhibited the growth

inhibition equal to the standard drug against ***E.coli, S.aureus* & *S.pyrogenus*** bacterial strains. The compound **11b, 11c, 11i, 12a, 12b, 12c & 12i** displayed promising activity against ***P.aeruginosa*** bacterial strains. Even in case of the fungal activity assay of the compound, **11h** exhibited the good activity against ***C. albicans*** fungal strain. The other compounds have shown good to moderate activity against bacterial and fungal strains.

Table II Physical, characterization data of compound (11a-j) & (12a-j)

Comp.	Functional Group	Mol. Formula	Mol. wt.	Yield (Time/hrs)	%C Required (Found)	%H Required (Found)	%N Required (Found)
11a & 12a	-H	$C_{15}H_{13}N_4ClS$	316.80	58% (5-6)	56.87 (56.85) & (56.86)	4.14 (4.12) & (4.10)	17.68 (17.65) & (17.66)
11b & 12b	2-Cl	$C_{15}H_{12}N_4Cl_2S$	351.25	49% (5-6)	51.29 (51.27) & (51.26)	3.44 (3.41) & (3.42)	15.95 (15.92) & (15.94)
11c & 12c	4-Cl	$C_{15}H_{12}N_4Cl_2S$	351.25	51% (5-6)	51.29 (51.25) & (51.24)	3.44 (3.43) & (3.41)	15.95 (15.94) & (15.92)
11d & 12d	2-OCH_3	$C_{16}H_{15}N_4OClS$	346.83	61% (5-6)	55.41 (55.39) & (55.40)	4.36 (4.32) & (4.33)	16.15 (16.14) & (16.13)
11e & 12e	3-OCH_3	$C_{16}H_{15}N_4OClS$	346.83	58% (5-6)	55.41 (55.40) & (55.38)	4.36 (4.34) & (4.31)	16.15 (16.15) & (16.12)
11f & 12f	4-OCH_3	$C_{16}H_{15}N_4OClS$	346.83	60% (5-6)	55.41 (55.41) & (55.39)	4.36 (4.32) & (4.32)	16.15 (16.13) & (16.10)
11g & 12g	2-NO_2	$C_{15}H_{12}N_5O_2ClS$	361.80	55% (5-6)	49.79 (49.75) & (49.76)	3.34 (3.33) & (3.31)	19.36 (19.33) & (19.32)
11h & 12h	4-CH_3	$C_{16}H_{15}N_4ClS$	330.83	58% (5-6)	58.09 (58.09) & (58.00)	4.57 (4.52) & (4.55)	16.93 (16.90) & (16.92)
11i & 12i	3,5-di- CH_3	$C_{17}H_{17}N_4ClS$	344.86	61% (5-6)	59.21 (59.18) & (59.19)	4.97 (4.94) & (4.95)	16.25 (16.22) & (16.22)
11j & 12j	-$NHCOCH_3$	$C_{17}H_{16}N_5OClS$	373.85	52% (5-6)	54.61 (54.60) & (54.58)	4.31 (4.29) & (4.30)	18.73 (18.71) & (18.70)

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Table III Comparison of compound (11a-j) & (12a-j)

Comp.	Specific Optical Rotation	Melting Point	Comp.	Specific Optical Rotation	Melting Point
11a	-80°	123-126 ℃	**12a**	20°	129-136 ℃
11b	-130°	105-110 ℃	**12b**	20°	74-88 ℃
11c	-140°	135-140 ℃	**12c**	20°	89-98 ℃
11d	-100°	145 ℃ dec.	**12d**	70°	105-115 ℃
11e	-110°	98-104 ℃	**12e**	70°	126-130 ℃
11f	-120°	107-114 ℃	**12f**	40°	122-125 ℃
11g	-130°	110-114 ℃	**12g**	50°	69-78 ℃
11h	-80°	115-130 ℃	**12h**	-	133-141 ℃
11i	-	170-177 ℃	**12h**	-	257-271 ℃
11j	-20°	132-137 ℃	**12j**	60°	181-194 ℃

CONCLUSION

In conclusion, we have described the synthesis and biological activities of a new 1,2,4-triazole derivative. These 1,2,4-triazole derivative showed lower antifungal activity than parent nystatin, while compound **11a** exhibited the growth inhibition almost equal to the standard drug against all bacterial strains.

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