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Synthesis, characterization and antimicrobial activity of novel biphenyl tetrazoles

Somisetti Narender Rao[1], T. Ravisankar [1], J. Latha [a]nd K. Sudhakar Babu*[3]

[1]Department of Research and Development, Srini Pharmaceuticals Ltd., Plot No.10, Type-C, Road No.8, Film nagar, Jubilee Hills, Hyderabad , Andhra Pradesh, India,
[2] College of Engg & Technology, Sri Krishna Devaraya University, Anantapur, A.P, India
[3]Department of Chemistry, Sri Krishna Devaraya University, Anantapur, A.P, India*

ABSTRACT

*A series of novel biphenyl tetrazoles compounds have been prepared from the secondary amides by the reaction with phosphorous pentachloride and sodium azide. These tetrazoles compounds were subjected to benzylic bromination and further condensed with n-methyl piperazine to afford compounds **6a-j** and **7**. The structures of the newly synthesized compounds were characterized by NMR, Mass, IR spectral data and elemental analysis. All the synthesized compounds were screened for their antibacterial activity.*

Key words: Substituted tetrazoles, bromination, spectral data and antimicrobial activity.

INTRODUCTION

Tetrazole and its derivatives have attracted much attention because of their unique structure and applications as antihypertensive, antialergic, antibiotic and anticonvulsant agents [1-8]. Development of tetrazole chemistry has been largely associated with wide scale of applications of these classes of compounds in medicine, biochemistry, agriculture [9-12] and also a large number of medicinally important tetrazole heterocyclic incorporated drugs approved by the FDA [13-14]. The medicinal activity of tetrazole functionality is due to its ability to serve as bioequivalent (bioisostere) of the carboxylic acid group. 1, 5-disubstituted tetrazoles can be used as isosteres of the *cis*-amide bond of peptides [15-17]. Biphenyl tetrazole compounds play important role in the medicinal chemistry. Losartan was described as the first non-peptide AT1 receptor antagonist and the coined group name was sartans [18-19]. Most of these compounds share the biphenyl tetrazole unit or replacements thereof with the original advanced lead Losartan [20]. All these sartan drugs contain some common structural features represented by a biphenyl fragment bearing an acidic moiety (i.e.: tetrazole, carboxylic- or sulphonamidocarboxyl- group), linked to a hetero aromatic or acyclic system by means of a methylene group.

Piperazines are a broad class of chemical compounds with many important pharmacological properties. These compounds have remarkable pharmacological activities like antipsychotic, antimalarial, anticonvulsant, antiarrhythmic, antimicrobial, antioxidant and cytotoxic activities [21]. Slight change in the substitution pattern in piperazine nucleus causes distinguishable differences in their pharmacological activities [22].

Prompted by the various biological activities of tetrazole and its substituted derivatives, the author envisioned his approach towards the synthesis of a novel series of 1, 5-substituted biphenyl tetrazole derivatives. Similar to sartan series drugs, the author designed the targeted molecules having biphenyl tetrazole moiety which are linked to N-methyl piperazine through methylene group. The prime objective of the present study was to synthesize, characterize new biphenyl tetrazoles derivatives and evaluation of their antibacterial activity.

MATERIALS AND METHODS

All solvents and reagents were purchased from the commercial suppliers and used without further purification. All non-aqueous reactions were performed in dry glassware under an atmosphere of dry nitrogen. Organic solutions were concentrated under reduced pressure. Thin layer chromatography (TLC) was performed on Merck precoated Silica-gel 60F254 plates. The NMR was recorded in DMSO-d_6, $CDCl_3$, D_2O, at 400 MHz on a Varian Gemini FT NMR spectrometer. The chemical shifts were reported in δ ppm relative to TMS. The FT-IR spectra were recorded in the solid state as KBr dispersion using a Perkin-Elmer 1650 FT-IR spectrophotometer. The mass spectrum (70 eV) was recorded on an Agilent-6310 LC-MS spectrometer. The elemental analysis was carried out using Elementar Vario Micro analyzer.

Preparation of 4'-Methyl-biphenyl-2-carboxylic acid 2: To a stirred solution of compound **1** (100 g, 0.517 moles) in ethylene glycol (1.0 L) was added KOH (60.8, 1.086 moles) and heated to reflux. The contents were maintained under reflux for 15 hours. The completion of the reaction was monitored by TLC. The reaction mass was cooled to 25-30^{o}C and diluted with water (2.0 L) and washed with dichloromethane (2x 200 mL). The aqueous phase is separated and the pH was adjusted to 3-4 with 1N HCl and stirred for 3-4 hours at 25-30^{o}C. The precipitated product was filtered and washed with water (200 mL) and dried under vacuum at 60^{o}C for 10-12 hours to afford compound 2 as pale cream colored solid. Yield: 95 g (86.4%). M.P. 146-148 ^{o}C; IR (KBr): 3030 (m), 1697 (s); ^{1}H NMR: δ 2.39 (3H, s, CH_3), 7.17-7.42 (6H, m, Ar-H), 7.54 (1H, dt, J=7.4, 1.5, Ar-H) 7.92 (1H, dd, J= 7.7, 1.0, Ar-H).

The typical procedure for the preparation of titled compound 6a (Ar=phenyl series) starting from compound 2 is provided below and the same procedure was followed to prepare the remaining analogues.

Preparation of 4'-Methyl-biphenyl-2-carboxylic acid phenyl amide 3a: To a stirred solution of 4'-Methyl-biphenyl-2-carboxylic acid **2** (10g, 0.047 moles) in toluene (70 mL) was added pyridine (0.3 mL) and cooled the contents to 0-5^{o}C. Thionyl chloride (6.16 g, 0.051 moles) was added slowly drop wise during 10-15 minutes by maintaining the internal temperature 0-5^{o}C. The reaction mixture was slowly allowed to reach 25-30^{o}C and stirred for 2 hours at same temperature. The transformation of acid to acid chloride can be confirmed by TLC. After the completion of the reaction, the contents were cooled to 0-5^{o}C and added aniline (4.8 g, 0.052 moles) slowly drop wise during 10-15 minutes. The reaction mixture was allowed to reach 25-30^{o}C and maintained for 4 hours. After completion of the reaction, the solvent was distilled off completely under reduced pressure to afford residue. Added water (100mL) slowly to the residue and extracted with ethyl acetate (150 + 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ (50 mL) followed by water (50 mL). The organic phase was separated, dried over sodium sulphate (2.0g) and the solvent was distilled completely. To the residue, diisopropylether (20 mL) was added and again distilled completely to remove the traces of ethyl acetate. Finally diisopropylether (20 mL) was added and stirred the contents for 30-45 minutes at 25-30^{o}C followed by maintenance for 30-45 minutes at 0-5^{o}C. The separated compound was filtered and washed with precooled diisopropylether (10 mL). The wet cake was dried under vacuum at 40-45^{o}C for 8 hours to obtain compound **3a** as an off-white solid. Yield: 12.1 g (89.3%), M.P. 155^{o}C, Mass: 288.7 (M^+ +1), IR (KBr) (υ cm-1): 3226 (NH-amide), 2918 (C-H, alkyl), 1654 (C=O, amide), 1624 (C=C, aryl); ^{1}H NMR: δ 7.88 (d, 1H, J=7.6 Hz), 7.52-7.36 (m, 5H), 7.25-7.21(m, 3H), 7.13-7.11(m, 3H), 7.07-7.03(m, 1H), 2.38(s, 3H).

4'-Methyl-biphenyl-2-carboxylic acid (4-chloro-phenyl)-amide (3b): Yield: 85.5%, M.P. 163^{o}C, Mass: 322.4 (M^+ +1), IR (KBr) (υ cm-1): 3273 (NH-amide), 2919 (C-H, alkyl), 1650 (C=O, amide), 1631 (C=C, aryl); 7.85 (d,1H), 7.57-7.53 (m, 4H), 7.49-7.40(m, 3H), 7.34-7.32 (m, 3H), 6.93-9.85 (m, 1H), 2.41(s, 3H).

4'-Methyl-biphenyl-2-carboxylic acid (2-chloro-phenyl)-amide (3c): Yield: 87.6%, M.P. 158^{o}C,Mass: 322.6 (M^+ +1), IR (KBr) (υ cm-1): 3270 (NH-amide), 2919 (C-H, alkyl), 1652 (C=O, amide), 1628 (C=C, aryl); 8.39 (d, 1H), 7.80 (1d, H, J= 7.6 Hz), 7.58-7.40 (m, 4H), 7.38-7.34 (m, 2H), 7.28-7.23(m, 4H), 2.38(s, 3H)

4'-Methyl-biphenyl-2-carboxylic acid (2, 3-dichloro-phenyl)-amide (3d): Yield: 80.7%, M.P. 107^{o}C,Mass: 356.5, 357.2 (M^{+}, M^{+} +2), IR (KBr) (υ cm-1): 3305 (NH-amide), 2922 (C-H, alkyl), 1669 (C=O, amide), 1634 (C=C, aryl); ^{1}H NMR: δ 8.45 (d, 1H, J=8.0 Hz), 7.84(d, 1H, J= 7.6 Hz), 7.57-7.42 (m, 3H), 7.36-7.34(m, 2H), 7.26-7.23(m, 4H), 2.36(s, 3H).

4'-Methyl-biphenyl-2-carboxylic acid (3,4-dichloro-phenyl)-amide (3e): Yield: 82%, M.P. 147^{o}C, Mass: 356.4, 357.3 (M^{+}, M^{+} +2), IR (KBr) (υ cm-1): 3310 (NH-amide), 2913 (C-H, alkyl), 1665 (C=O, amide), 1636 (C=C, aryl); ^{1}H NMR: δ 7.88 (d, 1H, J=7.6 Hz), 7.57-7.53(m, 3H), 7.49-7.40(m, 2H), 7.34-7.32(m, 3H), 6.93-9.85(m, 2H), 2.41(s, 3H).

4'-Methyl-biphenyl-2-carboxylic acid (2-fluoro-phenyl)-amide (3f): Yield: 86%, M.P. 86^{o}C, Mass: 306.2 (M^{+} +1), IR (KBr) (υ cm-1): 3212 (NH-amide), 2919 (C-H, alkyl), 1651 (C=O, amide), 1613 (C=C, aryl); ^{1}H NMR: δ 8.33 (t, 1H, J= 8.0 Hz), 7.85(d, 1H, J= 7.6 Hz), 7.55-7.41(m, 3H), 7.37-7.28(m, 2H), 7.24-7.18(m, 2H), 7.12(t, 1H, J=15.6 Hz), 7.00-6.92 (m, 2H), 2.36(s, 3H).

4'-Methyl-biphenyl-2-carboxylic acid (2,5-difluoro-phenyl)-amide (3g): Yield: 72%, M.P. 103^{o}C, Mass: 324.2 (M^{+} +1), 3197 (NH-amide), 2925 (C-H, alkyl), 1650 (C=O, amide), 1625 (C=C, aryl); ^{1}H NMR: δ 8.24-8.19 (m, 1H), 7.85 (d, 1H, J=7.6 Hz), 7.56-7.53(m, 1H), 7.48-7.40(m, 2H), 7.33-7.29 (m, 2H), 7.29-7.25(m, 2H), 7.22-7.20 (m, 2H), 6.90-6.84(m, 1H), 6.68-6.62(s, 1H), 2.36 (s, 3H).

4'-Methyl-biphenyl-2-carboxylic acid (2,3,4-trifluoro-phenyl)-amide (3h): Yield: 68.7%, M.P. 147^{o}C, Mass: 342.2 (M^{+} +1); 3234 (NH-amide), 2929 (C-H, alkyl), 1641 (C=O, amide), 1613 (C=C, aryl); ^{1}H NMR: δ 8.01 (m, 1H), 7.85 (d, 1H, J=7.6 Hz)), 7.60-7.40(m, 3H), 7.34-7.30 (m, 2H), 7.25-7.18 (m, 1H), 7.16-7.08 (s, 1H).

4'-Methyl-biphenyl-2-carboxylic acid benzyl amide (3i): Yield: 76%, M.P. 145^{o}C, Mass: 302.3 (M^{+} +1), IR (KBr, cm-1) 3293 (NH-amide), 2919 (C-H, alkyl), 1648 (C=O, amide), 1617 (C=C, aryl); ^{1}H NMR: δ 7.73 (dd, 1H, J=7.6, J=1.2 Hz), 7.48-7.28 (m, 5H), 7.23-7.15(m, 5H), 6.90-6.87(m, 2H), 5.44(s, 1H, br), 4.34(s, 2H), 2.39(s, 3H).

Preparation of 5-(4'-Methyl-biphenyl-2-yl)-1-phenyl-1H-tetrazole 4a: To a stirred solution of compound **3a** (7g, 0.024 moles) in carbon tetrachloride (70 mL) was added phosphorous pentachloride (9 g, 0.043 moles) under Nitrogen atmosphere. The reaction mixture was heated to reflux and maintained for 3 hours. After completion of the reaction (amide to iminoyl chloride), the solvent was distilled off completely under reduced pressure. The residue was cooled to 0-5^{o}C, added DMF (70 mL) under Nitrogen atmosphere and stirred for 10-15 minutes to get clear solution. A suspension of sodium azide (4.2 g, 0.065 moles) in DMF (70 mL) was cooled to 0-5^{o}C. The iminoyl chloride solution in DMF was taken into the addition funnel and added to the suspension of sodium azide in DMF at 0-5^{o}C during 1 hour. After the addition, cooling was removed and stirred at 25-30^{o}C for overnight. The completion of the reaction can be monitored by TLC. The reaction mass was cooled to 0-5^{o}C and added water (50 mL) slowly during 20-30 minutes and maintained for 30-45 minutes at same temperature. The separated precipitate was flittered and washed with water (15 mL). The wet cake was dried under vacuum at 40-45^{o}C for 10 hours to obtain compound **4a** as off-white solid. Yield: 5.4 g (71%).M.P. 142^{o}C, Mass: 313.3 (M^{+} +1), IR (KBr) (υ cm-1): 2919 (C-H, alkyl), 1654 (C=C, aryl), 1496 (-N=N-

, tetrazole ring), 1331 (-C=N- , tetrazole ring), 1268, 1095 (-CN_4 , tetrazole ring); ^{1}H-NMR: δ 7.88-7.84 (m, 1H), 7.66-7.56(m, 2H), 7.32-7.28(m, 2H), 7.14(td, 1H, J=7.6 Hz), 7.04(d, 2H, J =8 Hz), 6.62-6.59(m, 2H), 6.52(d, 2H, J=8 Hz), 2.41(s, 3H).

1-(4-Chloro-phenyl)-5-(4'-methyl-biphenyl-2-yl)-1H-tetrazole (4b): Yield: 68.5%, M.P. 120^{o}C, Mass: 347.5 (M^{+} +1), IR (KBr) (υ cm-1): 2922 (C-H, alkyl), 1602 (C=C, aryl), 1497 (-N=N- ,tetrazole ring), 1354 (-C=N- , tetrazole ring), 1266, 1092 (-CN_4 , tetrazole ring); ^{1}H NMR: δ 7.78 (d, 1H, J=8Hz), 7.59(t, 1H, J=8 Hz), 7.53(t, 1H, J=8 Hz), 7.32(d, 1H, J=8 Hz), 7.08(d, 2H, J=8 Hz), 6.86(d, 2H, J=8 Hz), 6.56(d, 2H, J=8 Hz), 6.59(d, 2H, J=8Hz), 2.25(s, 3H).

1-(2-Chloro-phenyl)-5-(4'-methyl-biphenyl-2-yl)-1H-tetrazole (4c): Yield: 65.3%, M.P. 130^{o}C, Mass: 347.6 (M^{+} +1), IR (KBr) (υ cm-1): 2924 (C-H, alkyl), 1599 (C=C, aryl), 1500 (-N=N-, tetrazole ring), 1356 (-C=N- , tetrazole ring), 1268, 1095 (-CN_4 , tetrazole ring); ^{1}H NMR: δ 7.65 (d, 1H), 7.51(t, 1H), 7.45(t, 1H), 7.37(d, 1H), 7.10-6.80(m, 4H), 6.54(d, 2H), 6.50(d, 2H), 2.25(s, 3H).

1-(2, 3-Dichloro-phenyl)-5-(4'-methyl-biphenyl-2-yl)-1H-tetrazole (4d): Yield: 70%, M.P. 132^{o}C, Mass: 381.3, 383.4 (M^{+}, M^{+} +2), IR (KBr) (υ cm-1): 2924 (C-H, alkyl), 1625 (C=C, aryl), 1509 (-N=N-, tetrazole ring), 1355 (-C=N- , tetrazole ring), 1263, 1099 (-CN_4 , tetrazole ring); ^{1}H NMR: δ 7.80 (d, 1H), 7.58(t, 1H), 7.42(t, 1H), 7.35(d, 1H), 7.28 (d, 1H), 7.24 (d, 1H), 6.99(d, 2H), 6.66-6.45 (m, 3H), 2.35(s, 3H).

1-(3,4-Dichloro-phenyl)-5-(4'-methyl-biphenyl-2-yl)-1H-tetrazole (4e): Yield: 68.5%, M.P. 135^{o}C, Mass: 381.4, 383.6 (M^{+}, M^{+} +2), IR (KBr) (υ cm-1): 2945 (C-H, alkyl), 1636 (C=C, aryl), 1510 (-N=N-, tetrazole ring), 1350 (-C=N- , tetrazole ring), 1265, 1095 (-CN_4 , tetrazole ring); ^{1}H NMR: δ 7.84 (d, 1H, J=6.4 Hz), 7.62(t, 1H, J=6.0 Hz), 7.56(t, 1H, J=6.0 Hz), 7.36(d, 1H, J=6.0 Hz)), 7.2(d, 1H, J= 7.2 Hz), 6.91(d, 2H, J=6.4 Hz), 6.66(d, 1H, J=4 Hz), 6.49-6.45(m, 3H), 2.33(s, 3H).

1-(2-Fluoro-phenyl)-5-(4'-methyl-biphenyl-2-yl)-1H-tetrazole (4f): Yield: 62.1%, M.P. 142^{o}C, Mass: 331.4 (M^{+} +1), IR (KBr) (υ cm-1): 2922 (C-H, alkyl), 1610 (C=C, aryl), 1505 (-N=N-, tetrazole ring), 1344 (-C=N- , tetrazole ring), 1264, 1066 (-CN_4 , tetrazole ring); ^{1}H NMR: δ 7.74 (d, 1H, J=8Hz), 7.54(t, 1H, J=4 Hz), 7.48(t, 1H, J=4 Hz), 7.29-7.24(m, 2H), 6.92-6.86(m, 4H), 6.55(d, 2H, J=4 Hz), 6.44(t, 1H, J=4 Hz), 2.30(s, 3H).

1-(2,5-Difluoro-phenyl)-5-(4'-methyl-biphenyl-2-yl)-1H-tetrazole (4g): Yield: 59%, M.P. 154^{o}C, Mass: 349.3 (M^{+} +1), IR (KBr) (υ cm-1): 2924 (C-H, alkyl), 1599 (C=C, aryl), 1513(-N=N-, tetrazole ring), 1350 (-C=N- , tetrazole ring), 1262, 1100 (-CN_4 , tetrazole ring); ^{1}H NMR: δ 7.78 (d, 1H, J=6Hz), 7.57(t, 1H, J=6 Hz), 7.51(t, 1H, J=6 Hz), 7.33(d, 1H, J=6 Hz)), 7.02-6.87(m, 4H), 6.56(d, 2H, J=6 Hz), 6.09(s, 1H, br), 2.33(s, 3H).

5-(4'-Methyl-biphenyl-2-yl)-1-(2,3,4-trifluoro-phenyl)-1H-tetrazole (4h): Yield: 58.7%, M.P. 109^{o}C, Mass: 367.3 (M^{+} +1), IR (KBr) (υ cm-1): 2924 (C-H, alkyl), 1625 (C=C, aryl), 1517 (-N=N-, tetrazole ring), 1311 (-C=N- , tetrazole ring), 1206, 1101 (-CN_4 , tetrazole ring); ^{1}H NMR: δ 7.69 (d, 1H, J=8Hz), 7.59(t, 1H, J=8 Hz), 7.53(t, 1H, J=8 Hz), 7.32(d, 1H, J=8 Hz), 6.88(d, 2H, J=8 Hz), 6.76-6.70(m, 3H), 6.60(d, 1H, J=8 Hz), 2.35(s, 3H).

1-Benzyl-5-(4'-methyl-biphenyl-2-yl)-1H-tetrazole (4i): Yield: 45%, M.P. 137^{o}C, Mass: 327.2 (M^{+} +1); IR (KBr) (υ cm-1): 2920 (C-H, alkyl), 1607 (C=C, aryl), 1510 (-N=N- , tetrazole ring), 1365 (-C=N- , tetrazole ring), 1270, 1100 (-CN_4 , tetrazole ring); ^{1}H NMR (DMSO-d_6): δ 7.74-7.70 (dd, 1H, , *J*=7.6 Hz, *J*=1.6 Hz), 7.59-7.50 (m, 3H), 7.27-7.19 (m, 3H), 7.09-7.08 (d, 2H, *J*=7.6 Hz), 6.89-6.87 (d, 2H, *J*=8.0 Hz), 6.80-6.79 (d, 2H, *J*=6.4 Hz), 5.08 (s, 2H), 2.28 (s, 3H).

Preparation of 5-(4'-Bromomethyl-biphenyl-2-yl)-1-phenyl-1H-tetrazole 5a: To a stirred solution of compound **4a** (4 g, 0.012 moles) in carbon tetrachloride (40 mL) was added DBDMH (2.4 g, 0.008 moles) and stirred for 10-15 minutes at 25-30^{o}C. Added a solution of benzoyl peroxide (0.155 g, 0.0006 moles) in carbon tetrachloride (5 mL) to the reaction mixture and the contents were refluxed for 6-7 hours. After the completion of the reaction, the contents were cooled to 25-30^{o}C and diluted with dichloromethane (35 mL). The contents were stirred at same temperature for 20-30 minutes, the undissolved salts were filtered and washed with dichloromethane (5 mL). The filtrate was washed with water (70 mL) and dried over anhydrous sodium sulphate (0.5 g). The solvent was evaporated completely under vacuum and co-distilled with diisopropylether (15 mL). To the residue added diisopropylether (35 mL) and stirred at 25-30^{o}C for 30-45 minutes followed by maintenance at 0-5^{o}C for 45 minutes. The product was filtered, washed with precooled diisopropylether (5 mL) and dried under vacuum at below 50^{o}C to afford compound **5a** as off-white solid. Yield: 4.1 g (82 %), M.P. 115^{o}C, Mass: 391.6, 393.2(M^{+}, M^{+} +2)), IR (KBr) (υ cm-1): 2956 (C-H, alkyl), 1598 (C=C, aryl), 1419 (-N=N- , tetrazole ring), 1319 (-C=N- , tetrazole ring), 1268, 1106 (-CN_4 , tetrazole ring), 611 (C-Br); ^{1}H NMR ($CDCl_3$) δ 7.90-7.50 (m, 4H), 7.40-6.90 (m, 4H), 6.70-6.45 (m, 5H), 4.45 (s, 2H).

.5-(4'-Bromomethyl-biphenyl-2-yl)-1-(4-chloro-phenyl)-1H-tetrazole (5b): Yield: 76.2%, M.P. 116^{o}C, Mass: 426.0, 427.4(M^+, M^+ +2); IR (KBr) (υ cm-1): 2959 (C-H, alkyl), 1601 (C=C, aryl), 1495 (-N=N- , tetrazole ring), 1352 (-C=N- , tetrazole ring), 1266, 1092 (-CN_4 , tetrazole ring), 835 (Ar-Cl), 610 (C-Br); ^{1}H NMR ($CDCl_3$): δ 7.9-7.83 (m, 1H), 7.68-7.57 (m, 2H), 7.38-7.06 (m, 5H), 6.61-6.53 (m, 4H), 4.43(s, 2H).

5-(4'-Bromomethyl-biphenyl-2-yl)-1-(2-chloro-phenyl)-1H-tetrazole (5c): Yield: 77.5 %, M.P. 158^{o}C, Mass: 426.1, 427.6(M^+, M^+ +2), IR (KBr) (υ cm-1): 2956 (C-H, alkyl), 1604 (C=C, aryl), 1485 (-N=N- , tetrazole ring), 1356 (-C=N- , tetrazole ring), 1270, 1098 (-CN_4 , tetrazole ring), 814 (Ar-Cl), 609 (C-Br); ^{1}H NMR ($CDCl_3$): δ 7.84-7.78 (m, 1H), 7.62-7.49 (m, 2H), 7.39-7.05 (m, 6H), 6.76-6.70 (m, 2H), 6.28-6.23 (m, 1H), 4.48(s, 2H).

5-(4'-Bromomethyl-biphenyl-2-yl)-1-(2,3-dichloro-phenyl)-1H-tetrazole (5d): Yield: 68.7%, M.P. 128^{o}C, Mass: 461.2 (M^+ +1), IR (KBr) (υ cm-1): 2954 (C-H, alkyl), 1598 (C=C, aryl), 1509 (-N=N- , tetrazole ring), 1355 (-C=N- , tetrazole ring), 1263, 1099 (-CN_4 , tetrazole ring), 843 (Ar-Cl), 606 (C-Br); ^{1}H NMR ($CDCl_3$): δ 7.84-7.78 (m, 1H), 7.64-7.51 (m, 2H), 7.48-7.38 (m, 1H), 7.34-7.18(m, 3H), 7.07-6.98(m, 1H), 6.80-6.72 (m, 2H), 6.20-6.13(m, 1H), 4.50 (s, 2H).

5-(4'-Bromomethyl-biphenyl-2-yl)-1-(3,4-dichloro-phenyl)-1H-tetrazole (5e): Yield: 70 %, M.P. 133^{o}C, Mass: 461.5 (M^+ +1), IR (KBr) (υ cm-1): 2950 (C-H, alkyl), 1585 (C=C, aryl), 1495 (-N=N- , tetrazole ring), 1336 (-C=N- , tetrazole ring), 1265, 1095 (-CN_4 , tetrazole ring), 825 (Ar-Cl), 604 (C-Br); ^{1}H NMR ($CDCl_3$): δ 7.84-7.78 (m, 1H), 7.64-7.51 (m, 2H), 7.48-7.38 (m, 1H), 7.34-7.18(m, 3H), 7.07-6.98 (m, 1H), 6.80-6.72 (m, 2H), 6.20-6.13 (m, 1H), 4.50(s, 2H).

5-(4'-Bromomethyl-biphenyl-2-yl)-1-(2-fluoro-phenyl)-1H-tetrazole (5f): Yield: 75 %, M.P. 144^{o}C, Mass: 411.2 (M^+ +1), IR (KBr) (υ cm-1): 2945 (C-H, alkyl), 1599 (C=C, aryl), 1505 (-N=N- , tetrazole ring), 1344 (-C=N- , tetrazole ring), 1264, 1096 (-CN_4 , tetrazole ring), 1224 (Ar-F), 606 (C-Br); ^{1}H NMR ($CDCl_3$): δ 7.85-7.79 (m, 1H), 7.66-7.56 (m, 2H), 7.39-7.17 (m, 3H), 7.08-6.88(m, 2H), 6.73-6.59 (m, 2H), 6.25-6.15 (m, 1H), 4.50 (s, 2H).

5-(4'-Bromomethyl-biphenyl-2-yl)-1-(2,5-difluoro-phenyl)-1H-tetrazole (5g): Yield: 72.3%, M.P. 138^{o}C, Mass: 428.5 (M^+ +1), IR (KBr) (υ cm-1): 2950 (C-H, alkyl), 1699 (C=C, aryl), 1508 (-N=N- , tetrazole ring), 1349 (-C=N- , tetrazole ring), 1256, 1095 (-CN_4 , tetrazole ring), 1193 (Ar-F), 609 (C-Br); ^{1}H NMR ($CDCl_3$): δ 7.85-7.79 (m, 1H), 7.66-7.56 (m, 2H), 7.39-7.17 (m, 3H), 7.08-6.88(m, 2H), 6.73-6.59 (m, 2H), 6.25-6.15 (m, 1H), 4.50(s, 2H).

5-(4'-Bromomethyl-biphenyl-2-yl)-1-(2,3,4-trifluoro-phenyl)-1H-tetrazole (5h): Yield: 62.5%, M.P. 135^{o}C, Mass: 445.6 (M^+ +1), IR (KBr) (υ cm-1): 2984 (C-H, alkyl), 1624 (C=C, aryl), 1509 (-N=N- , tetrazole ring), 1346 (-C=N- , tetrazole ring), 1254, 1093 (-CN_4 , tetrazole ring), 1230 (Ar-F), 611 (C-Br); ^{1}H NMR ($CDCl_3$): δ 7.85-7.55 (m, 3H), 7.39-7.16 (m, 4H), 6.90-6.68 (m, 3H), 6.30-6.22 (m, 1H), 4.48(s, 2H).

1-Benzyl-5-(4'-bromomethyl-biphenyl-2-yl)-1H-tetrazole (5i): low melting semi solid, Yield: 55%, low melting semi solid, MS (m/z): 405.3, 407.5 (M^+, M^+ +2); ^{1}H NMR (DMSO d_6): δ 7.76-7.72 (t, 1H, *J*=7.4 Hz), 7.76-7.48 (m, 3H), 7.44-733 (m, 2H), 7.25-7.18 (m, 3H), 7.01-6.92 (m, 2H), 6.81 (d, 2H *J*=6.82 Hz), 5.14 (s, 2H), 4.68 (s, 2H).

Preparation of 1-Methyl-4-[2'-(1-phenyl-1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-piperazine hydrochloride 6a: To a stirred solution of compound **5a** (2 g, 0.005 moles) in THF (20 mL) was added N-methyl piperazine (5 mL) and stirred the contents at 25-30^{o}C for overnight. The reaction mixture was observed to homogenous initially and the solid separation observed after maintaining for 1 hour (N-methyl piperazine hydrochloride). The reaction completion was confirmed by TLC and the solvent was distilled off completely under vacuum to obtain semi solid. Water (20 mL) and ethylacetate (50 mL) were added to the semisolid and stirred for 15-20 minutes. The layers were separated and the organic layer was washed with water (2x20 mL). Again water (50 mL) was added to organic layer and acidified to a pH of 1-2 with 1N HCl solution. The aquoues layer was separated and washed with ethyl acetate (2x20 mL). The aqueous layer pH was again adjusted to 9-10 using 10% Na_2CO_3 solution and extracted the product with ethylacetate (2x 30 mL). The organic phase was separated and washed with water (20 mL0 followed by brine solution (20 mL). The solvent was evaporated from the organic layer and the crude compound was dissolved in isopropanol (20 mL). The pH was adjusted to 1-2 using HCl in isopropanol (IPA.HCl, 17%) and the separated solid

was stirred for 1-1.5 hours at 25-30°C. The solvent was distilled from the reaction mass to obtain solid. The solid was slurred in diisopropylether (20 mL) for 1 hours at 20-25°C, filtered and washed with diisopropylether (5 mL). The wet product was dried under vacuum at below 50°C for 5-6 hours to afford compound 6a as off-white solid. Yield: 1.5 g (65.6%), M.P. 253°C, Mass: 411.4 (M^+ +1), IR (KBr) (υ cm-1): 2958 (C-H, alkyl), 1598 (C=C, aryl), 1496 (-N=N-, tetrazole ring), 1370 (-C=N- , tetrazole ring), 1253, 1112 (-CN_4 , tetrazole ring), 1154, 1018 (C-N, piperazine);^{1}H NMR (DMSO-d_6): 7.90-7.80 (d, 1H), 7.8-7.6 (m, 2H), 7.4-7.2 (m, 6H), 6.7(d, 2H), 6.6 (d, 2H), 3.9(s, 2H), 3.8-3.2 (m, 8H), 2.8 (s, 3H); ^{13} C-NMR (D_2O, δ ppm): 154.6, 141.0, 140.8, 133.1, 132.9, 132.1, 131.8, 131.1, 130.6, 130.0, 129.4, 129.2, 127.5, 123.6, 121.8, 60.3, 50.7, 48.8, 43.6.

1-{2'-[1-(4-Chloro-phenyl)-1H-tetrazol-5-yl]-biphenyl-4-ylmethyl}-4-methyl-piperazine hydrochloride 6b: Yield: 75%, M.P. 258 °C(*dec*), Mass: 445.4 (M^+ +1), IR (KBr) (υ cm-1): 2956 (C-H, alkyl), 1633 (C=C, aryl), 1495 (-N=N-, tetrazole ring), 1368 (-C=N- , tetrazole ring), 1259, 1093 (-CN_4 , tetrazole ring), 1147, 1018 (C-N, piperazine), 841 (Ar-Cl); ^{1}H NMR (DMSO-d_6, δ ppm): 7.85-7.80 (d, 1H), 7.8-7.6 (m, 2H), 7.5-7.3(m, 5H), 6.8(d, 2H), 6.7 (d, 2H), 3.9(s, 2H), 3.8-3.2 (m, 8H), 2.8 (s, 3H); ^{13}C-NMR (D_2O, δ ppm): (DMSO-d_6, δ ppm): 153.7, 140.1, 134.3, 132.0, 131.7, 131.3, 130.1, 129.3, 128.4, 128.1, 125.4, 121.8, 60.4, 50.6, 48.7, 43.3.

1-{2'-[1-(2-Chloro-phenyl)-1H-tetrazol-5-yl]-biphenyl-4-ylmethyl}-4-methyl-piperazine hydrochloride 6c: Yield: 73.7%, M.P. 262°C(*dec*), Mass: 445.8 (M^+ +1), IR (KBr) (υ cm-1): 2974 (C-H, alkyl), 1633 (C=C, aryl), 1490 (-N=N-, tetrazole ring), 1354 (-C=N- , tetrazole ring), 1258, 1098 (-CN_4 , tetrazole ring), 1147, 1019 (C-N, piperazine), 823 (Ar-Cl); ^{1}H NMR (DMSO-d_6, δ ppm): 7.8-7.4 (d, 10H), 7.2(d, 2H), 4.25(s, 2H), 3.8-3.4 (m, 8H), 2.8 (s, 3H); ^{13} C-NMR (D_2O, δ ppm): (DMSO-d_6, δ ppm): 152.8, 135.3, 135.1, 134.8, 134.0, 130, 129.1, 128.2, 127.9, 127.6, 127.1, 126.9, 126.5, 126.3, 1236.1, 60.1, 51.1, 48.3, 43.0.

1-{2'-[1-(2,3-Dichloro-phenyl)-1H-tetrazol-5-yl]-biphenyl-4-ylmethyl}-4-methyl-piperazine hydrochloride 6d: Yield: 55.2%, M.P. 264 °C (*dec*), Mass: 479.6, 481.2 (M^+, M^+ +2), IR (KBr) (υ cm-1): 2976 (C-H, alkyl), 1602 (C=C, aryl), 1469 (-N=N-, tetrazole ring), 1367 (-C=N- , tetrazole ring), 1279, 1094 (-CN_4 , tetrazole ring), 1162, 1019 (C-N, piperazine), 824 (Ar-Cl); ^{1}H NMR (DMSO-d_6, δ ppm): δ 7.9 (d, 1H), 7.8-7.74 (m, 2H), 7.65(d, 1H), 7.4-7.33 (m, 3H), 6.97(s, 1H), 6.8 -6.6(m, 3H), 4.21 (s, 2H), 3.8-3.35 (m, 8H), 2.82 (s, 3H); ^{13} C-NMR (D_2O, δ ppm): 153.8, 135.4, 135.2, 134.1, 134.2, 130.6, 129.5, 129.0, 128.3, 127.8, 127.5, 127.1, 126.8, 126.5, 126.3, 60.3, 51.6, 48.1, 42.7.

1-{2'-[1-(3,4-Dichloro-phenyl)-1H-tetrazol-5-yl]-biphenyl-4-ylmethyl}-4-methyl-piperazine hydrochloride 6e: Yield: 58%, M.P. 230 °C, Mass: 479.4, 481.4 (M^+, M^+ +2); IR (KBr) (υ cm-1): 2925 (C-H, alkyl), 1640 (C=C, aryl), 1483 (-N=N-, tetrazole ring), 1352 (-C=N- , tetrazole ring), 1256, 1111 (-CN_4 , tetrazole ring), 1155, 1016 (C-N, piperazine), 835 (Ar-Cl); ^{1}H NMR (DMSO-d_6, δ ppm): 7.9 (d, 1H), 7.8-7.7 (m, 2H), 7.6(d, 1H), 7.4-7.3 (m,3H), 6.95(s, 1H), 6.8 (d, 1H), 6.65-6.6 (d, 2H), 4.2(s, 2H), 3.8-3.3 (m, 8H), 2.8 (s, 3H); ^{13} C-NMR (D_2O, δ ppm): 154.08, 135.5, 135.2, 134.3, 134.2, 130.7, 129.1, 128.9, 128.2, 127.5, 127.4, 127.1, 126.7, 126.1, 126.3, 60.0, 51.4, 49.3, 42.8

1-{2'-[1-(2-Fluoro-phenyl)-1H-tetrazol-5-yl]-biphenyl-4-ylmethyl}-4-methyl-piperazine hydrochloride 6f: Yield: 72.6%, M.P. 250°C, Mass: 429.5 (M^+ +1), IR (KBr) (υ cm-1): 2935 (C-H, alkyl), 1630 (C=C, aryl), 1510 (-N=N-, tetrazole ring), 1345 (-C=N- , tetrazole ring), 1254, 1099 (-CN_4 , tetrazole ring), 1220 (Ar-F), 1141, 1015 (C-N, piperazine); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.9 (d, 1H), 7.8-7.71 (m, 2H), 7.61(d, 1H), 7.4-7.31 (m, 4H), 6.8-6.75 (m, 2H), 6.5 -6.41(m, 2H), 4.2 (s, 2H), 3.8-3.3 (m, 8H), 2.8 (s, 3H); ^{13} C-NMR (D_2O, δ ppm): 162.5, 154.8, 135.6, 135.3, 135.1, 131.2, 130.9, 129.7, 129.1, 127.5, 127.1, 125.0, 118.5,118.1, 60.2, 51.2, 49.1, 43.5.

1-{2'-[1-(2,5-Difluoro-phenyl)-1H-tetrazol-5-yl]-biphenyl-4-ylmethyl}-4-methyl-piperazine hydrochloride 6g: Yield: 62.5%, M.P. 188 °C, Mass: 447.3 (M^+ +1), IR (KBr) (υ cm-1): 2926 (C-H, alkyl), 1625 (C=C, aryl), 1514 (-N=N-, tetrazole ring), 1347 (-C=N- , tetrazole ring), 1258, 1101 (-CN_4 , tetrazole ring), 1196 (Ar-F), 1150, 1010 (C-N, piperazine); ^{1}H NMR (DMSO-d_6, δ ppm): 7.9 (d, 1H), 7.8-7.7 (m, 2H), 7.59(d, 1H), 7.4-7.29 (m, 4H), 6.9-6.8 (m, 3H), 4.2 (s, 2H), 3.8-3.31 (m, 8H), 2.82 (s, 3H); ^{13} C-NMR (D_2O, δ ppm): 158.5, 158.2, 154.3, 135.5, 135.3, 135.0, 131.1, 130.9, 129.8, 129.1, 127.6, 127.1, 118.5, 118.2, 118.0, 117.7, 60.2, 52.1, 49.5, 42.4.

1-Methyl-4-{2'-[1-(2,3,4-trifluoro-phenyl)-1H-tetrazol-5-yl]-biphenyl-4-ylmethyl}-piperazine hydrochloride 6h: Yield: 47.3%, M.P. 248 °C, Mass: 465.4 (M^+ +1), IR (KBr) (υ cm-1): 2984 (C-H, alkyl), 1623 (C=C, aryl), 1517 (-N=N-, tetrazole ring), 1371 (-C=N- , tetrazole ring), 1247, 1103 (-CN_4 , tetrazole ring), 1207 (Ar-F), 1143,

1009 (C-N, piperazine); ^{1}H NMR (DMSO-d_6, δ ppm): 7.9 (d, 1H), 7.82-7.72 (m, 2H), 7.61(d, 1H), 7.41-7.3 (m, 4H), 6.9-6.82 (m, 2H), 4.2 (s, 2H), 3.8-3.31 (m, 8H), 2.82 (s, 3H); ^{13} C-NMR (D_2O, δ ppm): 154.4, 151.7, 151.3, 136.5, 135.4, 135.3, 135.1, 129.8, 129.1, 127.5, 127.0, 115.8, 115.5, 60.2, 50.7, 49.0, 43.4.

1-[2'-(1-Benzyl-1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-4-methyl-piperazine hydrochloride 6i: Yield: 41.5%, Mass: 425.4 (M^+ +1); ^{1}H NMR (DMSO-d_6, δ ppm): 7.9 (d, 1H), 7.8-7.7 (m, 2H), 7.59(d, 1H), 7.4-7.29 (m, 4H), 7.14-7.05 (m, 5H), 5.01(s, 2H), 4.2 (s, 2H), 3.8-3.28 (m, 8H), 2.8 (s, 3H); ^{13} C-NMR (D_2O, δ ppm): 154.4, 137.5, 135.8, 135.6, 135.0, 129.7, 129.0, 128.3, 127.6, 127.3, 127.1, 125.4, 60.90, 58.2, 50.5, 48.5, 42.94.

Preparation of 1-Methyl-4-[2'-(1-trityl-1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-piperazine 6j: To a stirred solution of compound **5j** (5 g, 0.009 moles) in THF (25 mL) was added N-methyl piperazine (15 mL) and stirred the contents at 25-30^{o}C for overnight. The reaction completion was confirmed by TLC and the solvent was distilled off completely under vacuum to obtain semi solid. Water (150 mL) was added to the semisolid and stirred for 15-20 minutes. The reaction mass pH was adjusted to 8-9 using 5% aqueous acetic acid and further stirred for 1-2 hours. The separated solid was filtered and washed with water (2x25 mL). The compound was dried under vacuum at 50^{o}C for 7-8 hours to afford compound 6j as off-white solid. Yield: 4.1 g (79.3%), M.P. 140^{o}C, Mass: 577.6 (M^+ +1), IR (KBr) (υ cm-1): 2933 (C-H, alkyl), 1596 (C=C, aryl), 1492 (-N=N-, tetrazole ring), 1368 (-C=N- , tetrazole ring), 1249, 1164 (-CN_4 , tetrazole ring), 1142, 1013 (C-N, piperazine); ^{1}H NMR (DMSO-d_6): δ 7.9 (d, 1H), 7.6-7.0 (m, 20H), 6.9 (d,2H), 3.4(s, 2H), 2.4-2.2 (m, 8H), 1.6 (s, 3H); ^{13}C-NMR (DMSO-d_6, δ ppm): 164.0, 142.1, 141.3, 139.9,136.9, 130,7,130.2,129.8, 129.0, 128.5, 128.2, 127.6, 128.2, 127.6, 127.3, 126.3, 82.8, 62.7, 55.1,53.2, 46.1

Preparation of 1-Methyl-4-[2'-(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-piperazine 7:
To a stirred solution of compound **6j** (2g) in toluene (15 mL) was added 32% aqueous HCl (10mL) and stirred the contents at 25-30^{o}C for 2-3 hours. Once the reaction completion was confirmed by monitoring TLC, the layers were separated and aqueous phase is washed with toluene (10 mL). The aqueous layer was separated and cooled to 10-15^{o}C. The pH of the aqueous layer was adjusted to 7-8 with 5% sodium hydroxide solution and extracted into dichloromethane (2x25 mL). The organic phase is separated, washed with brine solution (15 mL) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to obtain a residue compound. Added isopropanol (15 mL) to the residue and pH was adjusted to 1-2 with IPA.HCl and stirred for 45 minutes at 25-30^{o}C followed by maintenance at 0-5^{o}C for 30 minutes. The compound was filtered and washed with precooled isopropanol and dried under vacuum at 50^{o}C for 4-5 hours to afford compound 7 as white solid. Yield: 0.97 g (83.6%), M.P. 252^{o}C, Mass: 335.4 (M^+ +1), IR (KBr) (υ cm-1): IR (KBr) (υ cm-1): 2981 (C-H, alkyl), 1602 (C=C, aryl), 1481 (-N=N-, tetrazole ring), 1373 (-C=N- , tetrazole ring), 1278, 1101 (-CN_4 , tetrazole ring), 1156, 1016 (C-N, piperazine); ^{1}H NMR (DMSO-d_6): δ 7.9 (d, 1H), 7.8-7.4 (m, 3H), 7.1(s, 1H), 6.8 (d,2H), 6.6 (d, 1H), 4.25(s, 2H), 3.8-3.2 (m, 8H), 2.9 (s, 3H); ^{13} C-NMR (D_2O, δ ppm): 175.1,171.1,154.8,141.2,137.8, 136.8, 130.7,129.8, 128.0, 127.1, 126.9, 126.0, 122.3, 60.2, 48.9, 48.2, 45.6.

RESULTS AND DISCUSSION

In the present work, novel substituted biphenyl tetrazole compounds were prepared (**Scheme-1** and **Scheme-2**), characterized and evaluated for antimicrobial activity. The author has selected the commercially available 4'-Methyl-biphenyl-2-carbonitrile **1** as the starting compound for our synthesis. A series of novel biphenyl tetrazoles compounds have been prepared from the secondary amides by the reaction with phosphorous pentachloride and sodium azide. These tetrazoles compounds were subjected to benzylic bromination and further condensed with n-methyl piperazine to afford the targeted compounds **6a-j** and **7**. These novel compounds were screened antimicrobial activity.

The basic hydrolysis of 4'-Methyl-biphenyl-2-carbonitrile **1** using potassium hydroxide in ethylene glycol under reflux conditions afforded 4'-Methyl-biphenyl-2-carboxylic acid **2**. The amide compounds **3a-i** were prepared by reacting 4'-Methyl-biphenyl-2-carboxylic acid **2** with different aromatic amines. For this transformation, the acid compound was treated with thionyl chloride in presence of catalytic amount of pyridine in toluene medium to obtain the acid chloride and without isolation is further reacted with appropriate aniline compounds to obtain the amide compounds **3**. The reactions proceeded smoothly and gave the expected products **3a-i** in substantial yields (85-95%).

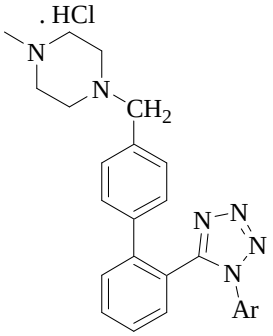


Figure.1: General structure of the novel biphenyl tetrazole compounds

*Where Ar= phenyl (**6a**), 4-chloro phenyl (**6b**), 2-chloro phenyl (**6c**), 2,3-dichloro phenyl (**6d**), 3,4-dichloro phenyl (**6e**), 2-fluoro phenyl (**6f**), 2,5-difluoro phenyl (**6g**), 2,3,4-trifluoro phenyl (**6h**), benzyl (**6i**), trityl (**6j**) and H(**7**).*

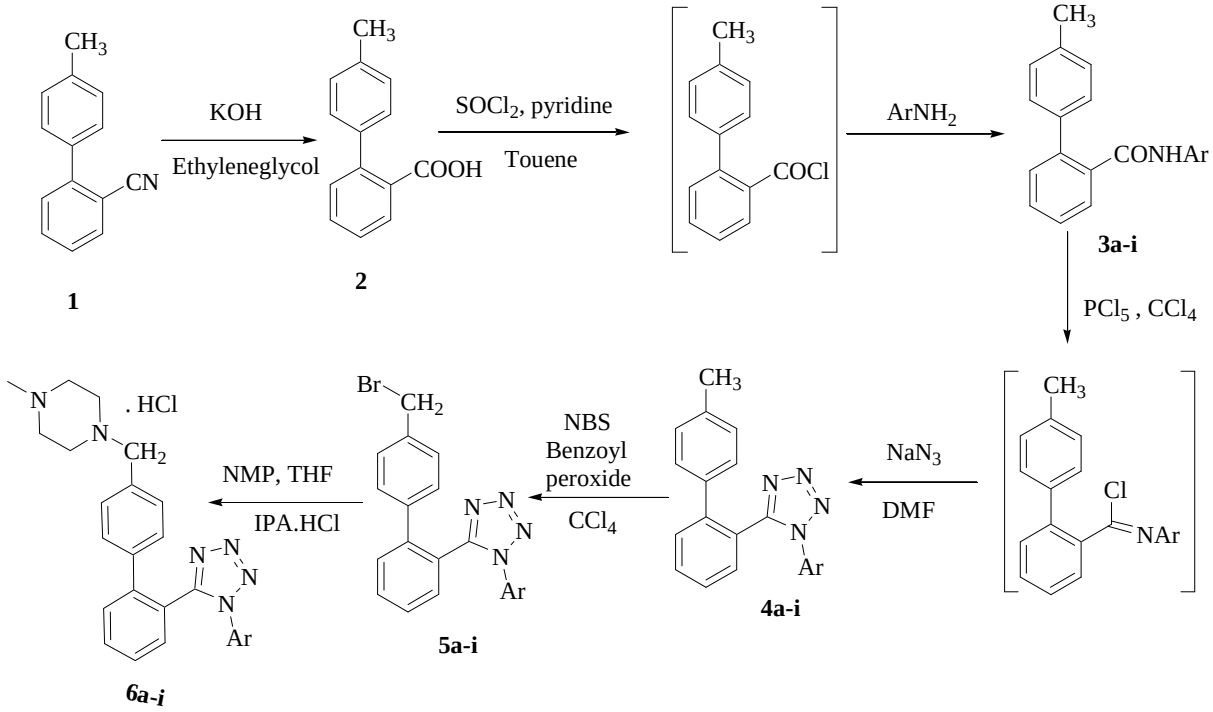
The structures of the compounds **3a-j** were established on the basis of IR, Mass and ^{1}H-NMR. The IR (KBr) spectra of 3a-i shows absorption bands 1645-1670 due to the C=O of amide bond and 3197-3310 due to the NH stretching of the amide bond. In the mass spectra, an appropriate molecular ion peak (M^+ +1) was obtained for all the amide derivatives from ESI–MS. For the compounds 3d and 3e, M^+ and M^+ +2 peaks were obtained due to the presence of dichloro substituents. All the amide derivatives have shown a characteristic doublet signal at 8.4-7.8 which is ascribable to the biphenyl ring aromatic proton adjacent to the amide substituent (-CONHAr). Another signal corresponding to halogen substitution at ortho position was observed at ~7.8 in the ^{1}H-NMR spectrum for the compounds **3c, 3d, 3f, 3g, 3h.** The signals corresponding to the remaining aromatic ring protons are observed at 6.6-7.5. NH proton of the amide is undetectable in NMR spectra. A singlet observed for the methyl protons at ~2.38. The expected signals with appropriate multiplicities for different types of protons were observed for the derivatives.

The amide compounds **3a-i** were treated with phosphorous pentachloride in carbon tetrachloride at reflux to obtain the intermediate imidoyl chloride, which were reacted with sodium azide in DMF at room temperature to afford the corresponding tetrazoles compounds **4a-i**. The yields for the tetrazoles formation were moderate (45-71%) and the products structure were confirmed by IR, Mass, ^{1}H-NMR.

The IR (KBr) spectra of 4a-i shows absorption bands 1490-1520 due to –N=N- and 1300-1400 due to -C=N- of tetrazole ring and two characteristic bands at 1200-1270 and 1090-1100 are due to the $–CN_4$ of tetrazole ring. In the mass spectra, an appropriate molecular ion peak (M^+ +1) was obtained for all the tetrazole derivatives from ESI–MS. For the compounds 4d and 4e, M^+ and M^+ +2 peaks were obtained due to the presence of dichloro substituents. All the tetrazole derivatives have shown a characteristic doublet signal at ~7.8 corresponding to the biphenyl ring aromatic proton adjacent to the tetrazole ring substitution. The signals corresponding to the remaining aromatic ring protons are observed at 7.6-6.5. A singlet observed for the methyl protons at ~2.3. The observed signals with appropriate multiplicities for different types of protons conformed the assigned structures of tetrazole derivatives **4a-i**.

The benzylic bromination on the compounds **4a-i** was accomplished using NBS in carbon tetrachloride under reflux. Dibenzoly peroxide was used as free radical initiator for this transformation. The dibromo impurity formation was observed in some of the analogues and the products were purified by recrystallising from diisopropylether. The yields are in the range of 55-85% and the products structure was confirmed by IR, Mass and NMR. The IR (KBr) spectra of **5a-h** shows absorption bands 1490-1520 Cm^{-1} due to –N=N- and 1300-1400 Cm^{-1} due to -C=N- of tetrazole ring and two characteristic bands at 1240-1280 and 1090-1115 are due to the $–CN_4$ of tetrazole ring. The bands at 600-615, about 835 and 1190-1230 are due to the halogens substituents. In the mass spectra, appropriate molecular ion peaks M^+, M^+ +1 and M^+ +2 were obtained depending on the halogen substituents from ESI–MS. All the bromo derivatives have shown a characteristic doublet signal at ~7.8 corresponding to the biphenyl ring aromatic proton adjacent to the tetrazole ring substitution. Aromatic protons showed multiplets in the range of 7.6-6.2. A singlet observed at ~4.4 in all the derivatives is attributed for the two benzylic protons.

The bromo compounds on condensation with N-methyl piperazine in THF gave the final compounds **6a-i** which were isolated in the form of hydrochloride salt (**Scheme-1**). The compound **6j** was prepared by reacting **5j** which is commercially available compound with N-methyl piperazine and compound **7** was prepared by the deprotection of compound **6j** in aqueous HCl (**Scheme-2**). These were characterized by IR, Mass, 1H-NMR, ^{13}C-NMR and elemental analysis. The IR (KBr) spectra of **6a-j** and **7** shows absorption bands 1490-1520 Cm^{-1} due to –N=N- and 1300-1400 Cm^{-1} due to -C=N- of tetrazole ring and two characteristic bands at 1240-1280 and 1090-1115 are due to the $–CN_4$ of tetrazole ring. About 1018 Cm^{-1} and 1140-1160 Cm^{-1} were characteristic for the piperazine ring. In the mass spectra, an appropriate molecular ion peak (M^+ +1) for free base was obtained for all the tetrazole derivatives from ESI–MS. For the compounds 6d and 6e, M^+ and M^+ +2 peaks were obtained due to the presence of dichloro substituents. All the final compounds have shown a characteristic doublet signal at ~7.9 corresponding to the biphenyl ring aromatic proton adjacent to the tetrazole ring substitution. The other aromatic protons showed signals in the range of 7.6-6.6. A characteristic singlet observed at ~4.2 in all the derivatives is attributed for the two benzylic protons. In all the synthesized compounds, the piperazine protons appeared at about 3.8 to 2.8 ppm integrated for 11 protons. A set of singlets at 5.01, 4.2 in the ^{1}H-NMR spectrum of the compound **6i** is attributed to the two benzylic groups attached to the piperazine ring and the tetrazole ring. In ^{13}C-NMR spectra of all synthesized compounds, tetrazole carbon signals were observed at about 175 to 152. The characteristic signal was observed at 175 for the compound **7** for tetrazole proton. This down field shift is due to the free tetrazole group. The aromatic carbon signals were observed in the range of 141 and 115. The peaks resonated at about 60 to 42 are attributed to the piperazine carbons.



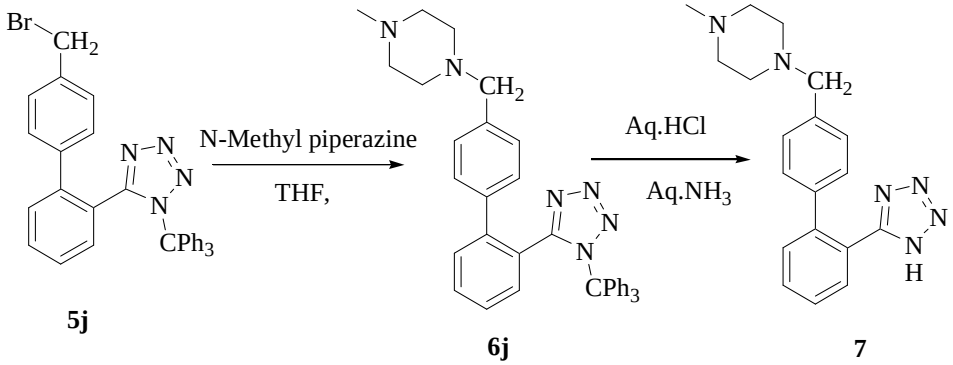
Scheme 1. General synthetic route for the preparation of the title compounds 6a-i.

Antimicrobial activity:

Determination of Minimum Inhibitory Concentration (MIC):

The *in vitro* antimicrobial activity of the compounds was tested by the tube dilution technique. Each of the test compounds and standard Gentamycin Sulfate were dissolved in 10% DMSO, at concentrations of 100 µg/mL. Further dilutions of the compounds and standards in the test medium were prepared at the required quantities of 1000, 500, 250, 125, 64.5, 32.2, 15.6, 7.8, 3.9 µg/mL concentrations. The final inoculums size was 105CFU/mL. The MICs were defined as the lowest concentrations of the compounds that prevented visible growth. A series of glass tubes containing different concentration of test compounds dissolved in DMSO and spiller in Nutrient Broth were incubated with one drop of inoculum and mixed gently by shaking the rack. Two growth control tubes were also prepared without the addition of test compound and its optical density was determined as follows. 0.1 ml of

control was mixed with 0.9 ml of Sterile Saline and with 0.2µL loop, an Agar plate was inoculated. The control contains 1 x 10^{-5} colony forming units/ml=20 colonies. Incubated the tubes for 24 hours at 37ºC in air. The turbidity produced in each tube was recorded by UV/Visible spectrophotometer. The turbidity produced by the Broth (without inoculums) was considered as 100% transparency. The minimum inhibitory concentration (MIC) was noted as the concentration of the test substance, which completely inhibits the growth of the microorganism i.e. 100% transparency. All the synthesized compounds were tested for their antibacterial activity (MIC-minimum inhibition concentration) against two gram positive bacteria Staphylococcus aureus and Bacillus subtilis and gram negative bacteria Escherichia coli and Salmonella typhi organisms. The results are depicted in **Table 1**.



5j **6j** **7**

Scheme 2. Synthetic route for the preparation of the title compounds 6j and 7.

Determination of Minimum Bactericidal concentration (MBC):
After completion of the incubatory period of MIC tubes, the solutions were are streaked on nutrient agar plates. These plates are incubated at 37^{o}C for 24 hours. The observed results are same as MIC values. The MBC data is provided in Table-1.

Table 1. Antimicrobial activity of synthesized compounds (minimum inhibitory concentration, MIC in µg/mL)

Comp. No.	Ar =	Gram positive Bacteria				Gram negative Bacteria			
		Staphylococcus aureus		Bacillus subtillis		Escherichia coli		Salmonella typhi	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
6a	**Phenyl**	**500**	**500**	**500**	**500**	**15.6**	**15.6**	**15.6**	**15.6**
6b	4-Chloro phenyl	1000	1000	1000	1000	125	125	125	125
6c	2-Chloro phenyl	1000	1000	1000	1000	125	125	125	125
6d	2,3-Dichloro phenyl	1000	1000	1000	1000	62.5	62.5	62.5	62.5
6e	3,4-Dichloro phenyl	1000	1000	1000	1000	31.2	31.2	31.2	31.2
6f	2-Fluoro phenyl	1000	1000	1000	1000	62.5	62.5	62.5	62.5
6g	2,5-Difluoro phenyl	1000	1000	1000	1000	31.2	31.2	31.2	31.2
6h	**2,3,4-Trifluoro phenyl**	**500**	**500**	**500**	**500**	**15.6**	**15.6**	**15.6**	**15.6**
6i	Benzyl	1000	1000	1000	1000	62.5	62.5	62.5	62.5
6j	Trityl	1000	1000	1000	1000	125	125	125	125
7	**H**	**500**	**500**	**500**	**500**	**15.6**	**15.6**	**15.6**	**15.6**
Gentamycin		500	500	500	500	7.8	7.8	7.8	7.8

From the above screening results it is evident that the new biphenyl tetrazole compounds were more active with respect to gram-positive bacteria and the activity is comparable with the standard compound Gentamicin Sulphate where as these compounds are observed to be less active with respect to the negative bacteria. Among all these compounds **6a, 6h** and **7** have shown significant activity and the remaining compounds shown moderate activity.

CONCLUSION

In conclusion, the author has successfully synthesized a novel series of biphenyl tetrazole compounds in moderate to good yields. The synthesized final compounds were characterized by the IR, Mass, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. As evident by the antimicrobial studies, these novel biphenyl tetrazole compounds were more active with respect to gram-positive bacteria (Staphylococcus aureus, Bacillus subtillis) and the compounds **6a**, **6h** and **7** have shown significant and comparable activity with the reference standard, Gentamycin sulfate. These data

may be more useful in future development of biphenyl tetrazole compounds as more potent and novel antimicrobial agents.

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