How to Cite:
Honmane, P. P., Singh, S. K., & Mohite, S. K. (2022). Design, synthesis and biological activity of 4-amino-5-(Benzimidazole-1-yl) triazole. *International Journal of Health Sciences*, *6*(S1), 10100–10113. https://doi.org/10.53730/ijhs.v6nS1.6882

Design, synthesis and biological activity of 4-amino-5-(Benzimidazole-1-yl) triazole

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***Abstract*---**Triazoles are an important class of nitrogen containing heterocycles, because many of them exhibit interesting biological activities, such as anti-inflammatory, antidepressant, antifungal, anticancer,anti-TB, analgesic and hypoglycemic. Sulfur-linked 1,2,4-triazoles represent an important group in lead compound discovery. Many bioactive sulfur-linked 1,2,4-triazole have been reported, such as antibacterial activity, antitumor activity, anti-HIV activity, anti-TMV (tobacco mosaic virus) activity, and so on. We report Synthesis and Biological activity of 4-amino-5-(Benzimidazole-1-yl) Triazole.

***Keywords*---**Benzimidazole, Anticancer, QSAR, Characterization, Pharmacologoical activity.

Introduction

The search for new agent is one of the most challenging tasks to the medicinal chemist. The synthesis of high nitrogen containing heterocyclic systems has been increasing over the past decade because of their utility in various applications such as propellants, explosives, pyrotechnics and especially chemotherapy. In recent years, the chemistry of triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. The derivatization of triazole is considered to be based on the phenomenon of bioisosterism in which replacement of oxygen of oxadiazole nucleus with nitrogen atom yields triazole analogue. There are two possible isomers of triazole [1, 2] depending on the position of nitrogen atom in the ring.

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
Manuscript submitted: 09 Feb 2022, Manuscript revised: 18 March 2022, Accepted for publication: 27 April 2022

Out of the two triazoles, 1,2,4-triazole have drawn great attention to medicinal chemists from two decades due to its wide variety of activity [3-7], low toxicity, good pharmacokinetic and pharmacodynamics profiles. 1,2,4-Triazole derivatives usually exist in solid form. [8-12]. 1,2,4-Triazole derivatives are readily soluble in polar solvents and only slightly soluble in nonpolar solvents, however, the solubility in non-polar solvents can be increased by substitution on the nitrogen atom. Due to annular prototropictautomerism, 1,2,4-triazoles without substituents on the ring nitrogen atoms, a priori can exist in three forms. Analysis of the crystal data [13] showed that with rare exceptions [14], unsymmetrical 3,5-disubstituited 1,2,4-triazoles exists in the solid state as tautomer bearing an electron substituent at position 3 and an electron-donor substituent at position 5. The precise experimental data on the annular tautomerism in 1,2,4-triazoles in solution are limited [15]. Tautomeric form with the hydrogen atom at N-1 adjacent to carbon atom bearing relatively less electronegative group was found to predominate in the solution.

Among the substituted 1,2,4-triazoles, 3-mercapto-1,2,4-triazoles exists in two tautomeric forms (3, 4), because the labile hydrogen may be attached either to the nitrogen or to the sulfur atom. It exhibits thione-thioltautomeric forms. 1, 2, 4-Triazole derivatives undergo Mannich reaction (Mannich reaction is a 3-component condensation reaction involving an active hydrogen containing formaldehyde and a secondary amine). The amino methylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds [16]. Amino group at position 4 of 1, 2, 4-triazole derivative undergoes reaction with 4-methoxybenzaldehyde [17] and eliminate water molecule with the formation of Schiff base. Compounds having Schiff base structure may exist as E/Z geometrical isomers about the –N=CH- double bond. The Z isomer can be stabilized in less polar solvents by an intra-molecular hydrogen bond. It is known that 1,2,4-triazole and 4,5- dihydro-1H triazol-5-one rings have weak acidic properties and so some 1,2,4-triazole and 4, 5-dihydro-1H derivatives were titrated potentiometrically with tetra-buty-lammonium hydroxide in non-aqueous solvents such as acetonitrile, isopropyl alcohol and N, N-dimethylformamide, and the half-neutralization potential values and the corresponding pKa values of the compounds were determined [18].

Triazoles are potential bioactive agents due to their wide spectrum of therapeutic importance. Drug molecules having 1, 2, 4-triazole nucleus with good activity.A triazole refers to any of the heterocyclic compound with molecular formula C2H3N3, having a five-membered ring of two carbon atoms and three nitrogen atoms. There are two sets of isomer that differ in the relative positions of the three nitrogen atoms. Each of these has two tautomers that differ by which nitrogen has a hydrogen bonded to it.

Triazoles are an important class of nitrogen containing heterocycles, because many of them exhibit interesting biological activities, such as anti-inflammatory, [1] antidepressant, [2] antifungal, [3-4] anticancer, [5-6] anti-TB, [7] analgesic [8] and hypoglycemic. [9] Sulfur-linked 1,2,4-triazoles represent an important group in lead discovery. Also many sulfur-linked 1, 2, 4-triazole have been reported, as antibacterial, [10-12] antitumor, anti-HIV, [13] and anti-TMV (tobacco mosaic virus) activities.[14] Thus pharmacologically important sulfur-linked 1,2,4-triazole have attracted attention of many researchers to pursue novel, and easy synthetic routes. There are very few routes available for synthesis of these molecules. Recently, the synthesis of 1,2,4-triazolidine-3-thiones has been reported using [C16MPy]AlCl3Br as reusable ionic liquid catalyst.[15] In another multi-component reaction 1,2,4-triazolidine-3-thione was synthesized from aldehyde, hydrazine hydrate, and trimethylsilylisothiocyanate (TMSNCS) using sulphamic acid as a catalyst.[16] In continuation to our research work on synthesis of heterocyclic molecules using novel methodology using hybridization.

Materials and Methods

Chemistry

Starting materials were purchased from commercial sources and were used without further purification. Solvents were dried according to standard procedure. The reaction progress was monitored by thin layer chromatography (TLC) on aluminium sheet obtained from Merck. Silica gel 60-120 mess was used for column chromatography. Melting points were recorded on Thermomik Campbell Melting Point apparatus having an oil bath system and were uncorrected. IR spectrum was recorded on FTIR (Perkin Elmer Spectrum RX1) by preparing KBrpellets.All^{1}H and ^{13}C NMR were recorded on 400 MHz Varian Mercury NMR instrument. All NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 solutions using TMS as an internal standard. Chemical shifts are reported in ppm (δ). Mass spectra were recorded on Agilent 7820A GC systems (SIS-Direct insertion probe) and LC-MS TOF 6520A. Melting points were measured on a Buchi B-545 melting point apparatus. IR spectra were recorded on “Perkin Elmer FTIR spectrometer-Spectrum RXI”. Solid were recorded as KBr pallets and liquids as thin film or $CHCl_3$. Chemical shift (δ) are given in ppm (parts per million) downfield from TMS used as an internal reference standard (δ_H = 0). Column chromatography was carried out with silica gel (Merck 60-120 mesh).

The synthesis of 4-amino-5-(Benzimidazole-1-yl) Triazole and its derivative has been progressed the following ways, the steps involved in this synthesis as follows,

Step 1st: Synthesis of Benzimidazole

A mixture of formic acid (11 mmol) and OPDA (10 mmol) was refluxed for 8 hrs. The reaction mixture was allowed to cool to room temperature and neutralized with 5N aqueous NaOH. The precipitate obtained was filtered and washed with water. Thus the product obtained was then characterized by H1 NMR, Mass and IR spectra

.Step 2nd: Synthesis of ethyl Benzimidazole 1-Acetate.

To a round bottom flask charged with actone 60 ml, 1H benzimidazole (0.10 mmol) and K_2CO_3 (0.30 mmol) were added to it. Then ethyl-choroacetate (0.12 mmol) was added to reaction mixture and allowed to reflux for 10 hrs. After completion of the reaction by checking with TLC, the reaction mixture was allowed to cool to room temperature. After cooling to room temperature, excess of acetone was removed under reduced vaccum and the precipitate was purified by column chromatography using ethyl acetate and hexane (20:80). Thus the product obtained was then characterized by H1 NMR, Mass and IR spectra.

Step 3: Synthesis of 2 Benzimidazole-1-yl acetohydrazide.

To a round bottom flask charged with ethanol 50ml, compound obtained from the (scheme 2) benzimidazole 1-acetate (0.1 mmol) and hydrazine hydrate (0.2 mmol) were added. The reaction mixture was allowed to reflux till the completion of the reaction. After completion of the reaction by checking with TLC, the solvent was removed from the reaction mixture using reduced pressure on rotavac. The reaction mixture was absorbed on the silica and subjected to column chromatography to obtain the pure compound using MeOH: DCM in the ratio 20:80. Thus the product obtained was then characterized by H1 NMR, Mass and IR spectra.

Step 4th: Synthesis of 2 Benzimidazole-1-yl Oxadiazole

An equimolar mixture of compound 2 Benzimidazole-1-yl acetohydrazide (0.001 mol) and phosphoryl oxy chloride with catalytic amount of sulphuric acid was refluxed for 10–16 h. Then reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds.

Step 5: Synthesis of 4-amino-5-(Benzimidazole-1-yl) Triazole

An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and substituted carboxylic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Then reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds shown in figure 3

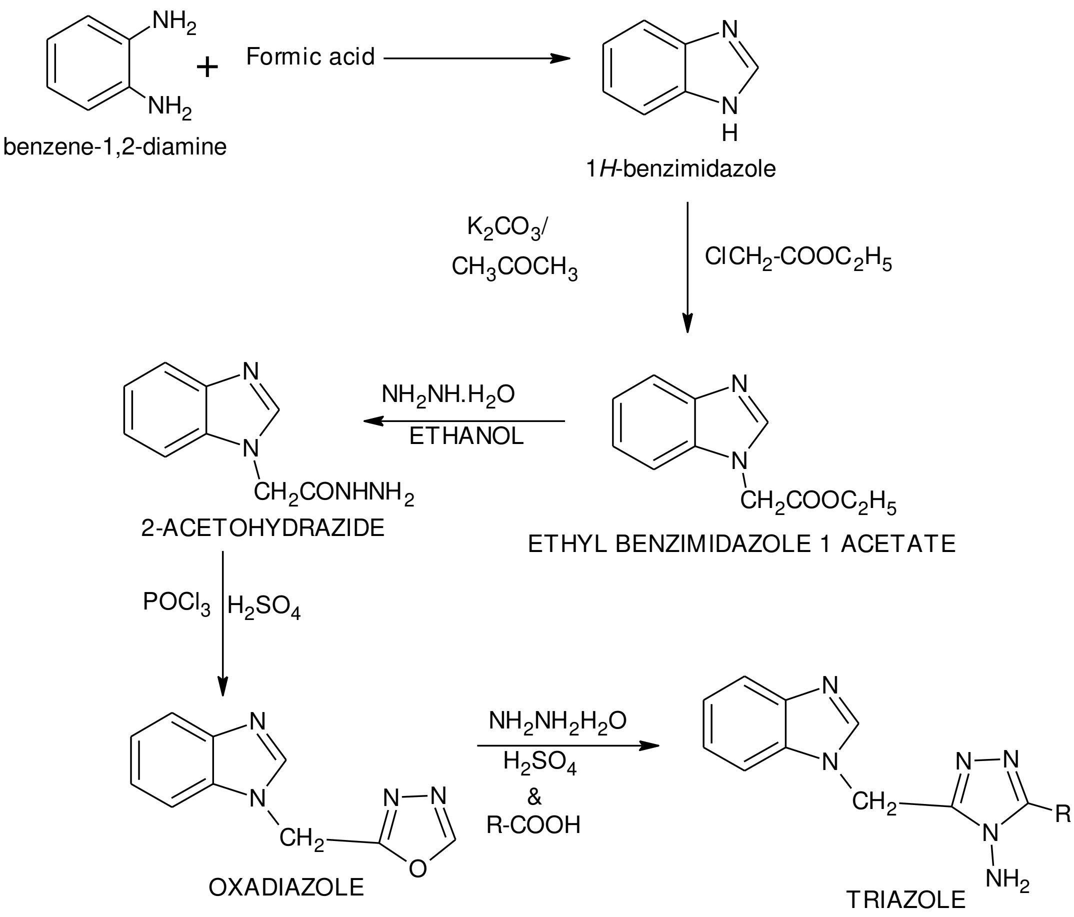


Figure 3. Scheme for synthesis of Triazole

General procedure for synthesis of 4-amino-5-(Benzimidazole-1-yl) Triazole (Table 6.1, entry 1):

An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and substituted carboxylic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Then reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds

3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine : (9a)

An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and formic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9a). White solid; m.p : 120°C, ^1H NMR (400 MHz, $CDCl_3$) δ 8.14(s, 1H) 7.84 (s, 2H), 7.69 – 7.64 (m, 2H), 7.36 (dd, J = 4.9, 1.7 Hz, 3H), 4.29

(q, *J* = 12.9, 6.3 Hz, 2H); FT-IR (KBr, cm^{-1}) 3447,3360, 2990, 2860, 1669, 1551, 1524, 1484, 1421, 1353, 1207, 1145, 1047, 1013, 953, 916, 875, 758, 690.MS : 215(M)$^{+}$ (m/z).

3-((1H-benzo[d]imidazol-1-yl)methyl)-5-methyl-4H-1,2,4-triazol-4-amine : (9b)

An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and ethanoic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9b). ^{1}H NMR (400 MHz, cdcl$_3$) δ 7.84 (s, 1H), 7.69 – 7.64 (m, 2H), 7.36 (dd, *J* = 4.9, 1.7 Hz, 2H), 5.5 (m, 2H), 4.29 (q, *J* = 12.9, 6.3 Hz, 2H), 1.32 (t, *J* = 6.1 Hz, 3H). FT-IR (KBr, cm^{-1}) 3470,3361, 2890, 2860, 1675, 1540, 1524, 14564, 1421, 1353, 1207, 1107, 1047, 1013, 950, 916, 875, 740, 690.MS **m/z: 229**

*3-((1H-benzo[d]imidazol-1-yl)methyl)-5-phenyl-4H-1,2,4-triazol-4-amine : (9c)*An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and benzoic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9c) ^{1}H NMR (400 MHz, dmso) δ 9.41 (q, 1H), 9.17 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 17.8, 7.3 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 1H), 7.72 – 7.57 (m, 4H), 4.06 (q, *J* = 10.5, 5.3 Hz, 2H), 1.20 (t, *J* = 4.2 Hz, 3H). FT-IR (KBr, cm^{-1}) 3447, 3360, 2985, 2860, 1670, 1541, 1530, 1484, 1421, 1340, 1207, 1169, 1047, 1013, 953, 946, 875, 758, 639.MSm/z: 290.13

*3-((1H-benzo[d]imidazol-1-yl)methyl)-5-(p-tolyl)-4H-1,2,4-triazol-4-amine :(9d)*An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and 4 methyl benzoic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9d). ^{1}H NMR (400 MHz, dmso) δ 9.41 (q, 1H), 9.17 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 17.8, 7.3 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 1H), 7.72 – 7.57 (m, 3H), 5.75-5.83 (d, *J* = 7.3 Hz, 2H) 4.06 (q, *J* = 10.5, 5.3 Hz, 2H), 1.20 (t, *J* = 4.2 Hz, 3H). FT-IR (KBr, cm^{-1}) 3447, 3360, 2990, 2860, 1669, 1551, 1524, 1484, 1421, 1353, 1207, 1145, 1047, 1013, 953, 916, 875, 758, 690.MSm/z: 304.14

*3-((1H-benzo[d]imidazol-1-yl)methyl)-5-(pyridin-4-yl)-4H-1,2,4-triazol-4-amine : (9e)*An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and isonicotinic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9e). ^{1}H NMR (400 MHz, dmso) δ 9.41 (q, 2H), 9.17 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 17.8, 7.3 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 1H), 7.72 – 7.57 (m, 3H), 5.75-5.83 (d, *J* = 7.3 Hz, 2H) 4.06 (q, *J* = 10.5, 5.3 Hz, 2H) m/z: 291.12

*3-((1H-benzo[d]imidazol-1-yl)methyl)-5-(4-nitrophenyl)-4H-1,2,4-triazol-4-amine : (9f)*An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and 4 nitrobenzoic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9f). ^{1}H NMR (400 MHz, dmso) δ 9.41 (q, 2H), 9.17 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 17.8, 7.3 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 1H), 7.72 – 7.57 (m, 3H), 5.75-5.83 (d, *J* = 7.3 Hz, 2H) 4.06 (q, *J* = 10.5, 5.3 Hz, 2H)m/z:335.11

*3-((1H-benzo[d]imidazol-1-yl)methyl)-5-benzyl-4H-1,2,4-triazol-4-amine : (9g)*An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and 2 phenylacetic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9g) ^{1}H NMR (400 MHz, dmso) δ 9.41 (q, 1H), 9.17 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 17.8, 7.3 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 1H), 7.72 – 7.57 (m, 4H), 4.06 (q, *J* = 10.5, 5.3 Hz, 4H), 1.20 (t, *J* = 4.2 Hz, 3H). FT-IR (KBr, cm^{-1}) 3447, 3360, 2985, 2860, 1670, 1541, 1530, 1484, 1421, 1340, 1207, 1169, 1047, 1013, 953, 946, 875, 758, 639m/z: 304.14

*3-((1H-benzo[d]imidazol-1-yl)methyl)-5-(4-methoxyphenyl)-4H-1,2,4-triazol-4-amine : (9h)*An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and 4 methoxy benzoic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9h) ^{1}H NMR (400 MHz, dmso) δ 9.41 (q, 1H), 9.17 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 17.8, 7.3 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 1H), 7.72 – 7.57 (m, 3H), 5.75-5.83 (d, *J* = 7.3 Hz, 2H) 4.06 (q, *J* = 10.5, 5.3 Hz, 2H), 1.20 (t, *J* = 4.2 Hz, 3H). FT-IR (KBr, cm^{-1}) 3447, 3360, 2990, 2860, 1669, 1551, 1524, 1484, 1421, 1353, 1207, 1145, 1047, 1013, 953, 916, 875, 758, 690.MSm/z: 320.14

*(E)-3-((1H-benzo[d]imidazol-1-yl)methyl)-5-styryl-4H-1,2,4-triazol-4-amine : (9i)*An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole (0.01 mol) and 4-methyl-2-nitrobenzoic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9i) ^{1}H NMR (400 MHz, dmso) δ 9.41 (q, 1H), 9.17 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 17.8, 7.3 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 1H), 7.72 – 7.57 (m, 3H), 5.75-5.83 (d, *J* = 7.3 Hz, 2H) 4.06 (q, *J* = 10.5, 5.3 Hz, 2H), 1.20 (t, *J* = 4.2 Hz, 3H). FT-IR (KBr, cm^{-1}) 3447, 3360, 2990, 2860, 1669, 1551, 1524, 1484, 1421, 1353, 1207, 1145, 1047, 1013, 953, 916, 875, 758, 690.MS349.35

*(E)-3-((1H-benzo[d]imidazol-1-yl)methyl)-5-(3-methoxystyryl)-4H-1,2,4-triazol-4-amine: (9j)*An equimolar mixture of compound 2 Benzimidazole-1-yl Oxadiazole

(0.01 mol) and 4-methoxy-2-nitrobenzoic acid in hydrazine hydrate (0.02 mol) with catalytic amount of sulphuric acid was refluxed for 10–12 h. Progress of the reaction was monitored on TLC. After completion of the reaction, reaction mixture was cooled, poured into ice-cold water and neutralized with 20% NaHCO3 solution. The resultant solid was filtered, washed with water and purified by column chromatography to give the title compounds (9j)^{1}H NMR (400 MHz, dmso) δ 9.41 (q, 1H), 9.17 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 17.8, 7.3 Hz, 2H), 8.04 (d, *J* = 7.4 Hz, 1H), 7.72 – 7.57 (m, 3H), 5.75-5.83 (d, *J* = 7.3 Hz, 2H) 4.06 (q, *J* = 10.5, 5.3 Hz, 2H), 1.20 (t, *J* = 4.2 Hz, 3H). FT-IR (KBr, cm^{-1}) 3447, 3360, 2990, 2860, 1669, 1551, 1524, 1484, 1421, 1353, 1207, 1145, 1047, 1013, 953, 916, 875, 758, 690.MSm/z: 346.15.

Results and Discussions

Protocol for antitubercular evaluation (REMA assay)

ResazurinMicrotiter Assay (REMA), a colorimetric method for detecting drug activity against bacteria is based on the reduction of an oxidation-reduction indicator (Resazurin, Fig. 2.39). Activity is detected by a change in color of the oxidation-reduction indicator, which is directly proportional to the number of viable bacteria in the medium. Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) is a blue non-fluorescent and non-toxic dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. MIC of each drug is interpreted as the lowest concentration of the compound that prevents a change in color of the resazurin.

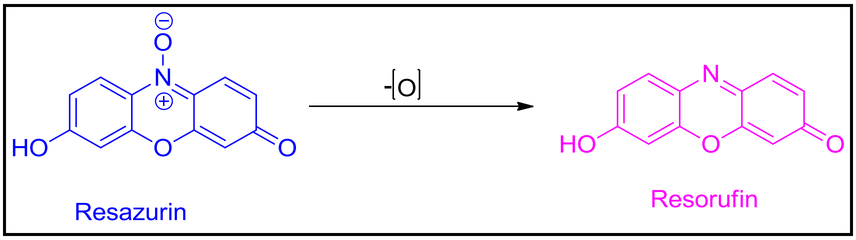


Fig. 1: Reduction of resazurin indicator

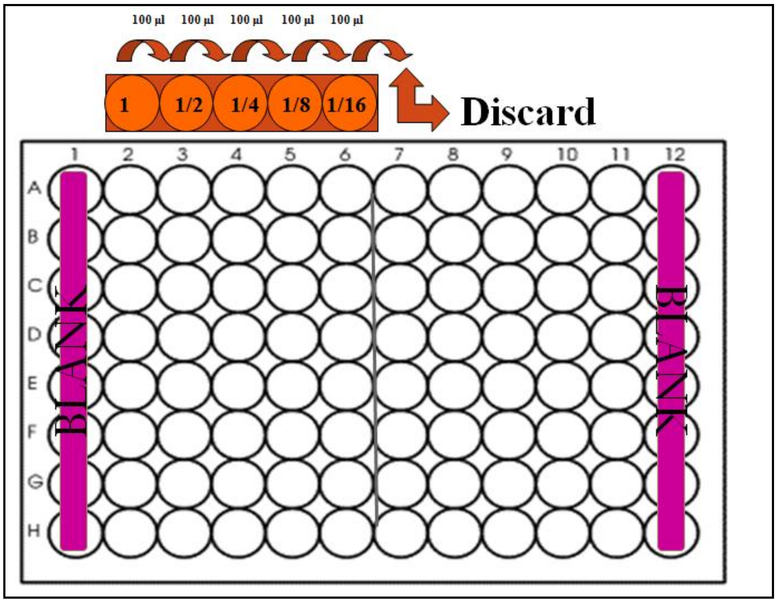


Fig. 2: Diagrammatic presentation of 96 well plate preparation

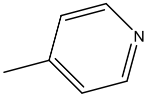
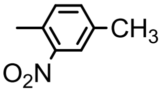
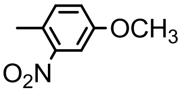
1.

All 10 novel **3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine** derivatives were evaluated for its *in-vitro* antibacterial activity against *M. tb* H37Rv strain using Resazurinmicrotitre plate assay (REMA) (Palomino et al., 2002). All compounds were evaluated for its antitubercular activity at a concentration of 1.56 to 50 µg/ml. The serial dilution technique was used to find out the minimum inhibitory concentration (MIC) of the synthesized benzimidazole derivatives. ResazurinMicrotiter Assay (REMA) protocol was used to determine the minimum inhibitory concentration (MIC) of all the synthesized 3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine derivatives. The synthesized compounds were screened against various baceria using serial dilution technique in middle brook 7H9 broth medium. Each compound (2 mg) was dissolved in 1 ml of DMSO. The serial dilution of each compounds were prepared using 96-well microtitre plate and 100 µl of bacterial cell suspension in nutrient media was added to each well. After 7 days of incubation, resazurin dye solution (0.02% w/v dissolved in distilled water) was added to each well and again incubated for 1 day. The MIC was determined by minimum concentration of compound that inhibits the growth of bacteria that is indicated by colour change from non-fluorescent blue to fluorescent pink colour. The MIC values were calculated by visual inspection for each well showing more than 90 % inhibition. Norfloxacin was used as the reference drug.

Antibacterial evaluation

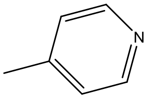
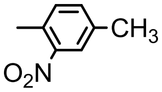
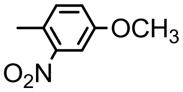
REMA protocol was used to determine the MIC of all the synthesized 3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine derivatives. The results of biological screening are summarized in (Table 1).From the MIC values obtained for synthesized 3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine derivatives, it was found that the compound shows moderate to good activities. It was also found that the substitution on the core molecule plays very important role in its antibacterial and antifungal activity. Compound having no substitution on the core (9a) and methyl substitution (9b) having moderate activity. It was also noted that phenyl substitution on the core moiety have least activity. The compound (9e) with isonicotinic acid derivative show very good activity. It is also important to note that the compounds having substituted phenyl derivative as (9h, 9i, 9j) also shows very good activity. This may be due to electron withdrawing and donating group on the same ring.

Table 1
Antibacterial activities of synthesized 3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine derivative

Sr. No	**Compound**	**R**	**[a]MIC (μg/mL)**
1	9a	H	6.25
2	9b	—CH_3	6.25
3	9c	—Ph	12.5
4	9d	—Ph—CH_3	12.5
5	9e		1.5
6	9f	—Ph−NO_2	6.25
7	9g	—CH_2·Ph	12.5
8	9h	—Ph−OCH_3	3.125
9	9i		3.125
10	9j		3.125
	Standdard		0.40

[a] MIC= minimum inhibitory concentration

Table 2
Antifungal activities of synthesized 3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine derivatives

Sr. No	**Compound**	**R**	**[a]MIC (μg/mL)**
1	9a	H	12.5
2	9b	—CH_3	25
3	9c	—Ph	12.5
4	9d	—Ph—CH_3	12.5
5	9e		3.25
6	9f	—Ph–NO_2	12.5
7	9g	—CH_2·Ph	12.5
8	9h	—Ph–OCH_3	6.25
9	9i		6.25
10	9j		3.125
	Standard		0.40

a

MIC= minimum inhibitory concentration

SAR of 3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine

From the biological screening of no substituted, alkyl, aroyl, nitro or alkoxy derivatives of triazole, it was found that triazole derivative with alkyl (methyl) substitution shows better activity than the aroyl (benzyl), substitution. Methyl substituted benzimidazole derivatives **9b** shown the activity range from 1.5 to 12.5 μg/mL, Introduction of electron donating (methyl) had a detrimental effect on the inhibitory activity compared to 9**a-b, 9d.** .Replacement of phenyl substitution with nicotinic substitution on triazole ring in the final designed molecule may be responsible for enhanced activity.

Table 3.
QikProp analysis data of synthesized 3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine

Title	#stars[a]	QPlogPo/w[b]	QPPCaco[c]	% Human oral absorption[d]	No of variation[e]
9a	2	4.693	781.498	100	0
9b	3	4.827	781.556	100	0
9c	2	5.075	781.541	95.482	1
9d	3	5.185	781.505	96.126	1
9e	3	5.317	781.582	96.896	1
9f	1	4.439	781.548	100	0
9g	3	5.164	781.454	96.002	1
9h	2	5	779.383	95.021	1
9i	1	5.136	781.867	95.841	1
9j	1	5.495	781.819	100	1

[a] #Stars: #stars are MW, dipole, IP, EA, SASA, FOSA, FISA, PISA, WPSA, PSA, volume, donorHB, accptHB, QPlogPoct, QPlogPw, QPlogPo/w, logS, QPLogKhsa, QPlogBB. The range predicted for this parameter using QikProp is 0–5; where 0–1 indicates no violation or best candidate.
[b] QPlogPo/w: This gives the predicted octanol/water partition coefficient. The range predicted for this parameter using QikProp is - 2.0–6.5.
[c] QPPCaco: QikProp predictions are for nonactive transport, where <25 is considered poor and >500 is considered excellent.
[d] % Human-Oral Absorption: This gives the predicted human oral absorption on 0–100% scale where >80% is considered high and <25% is considered

[e] No of variation from rule of five: This property denotes the number of violations of Lipinski's rule of five

QikProp analysis

From the QikPropanalysis of designed molecules, it was observed that the designed molecules exhibited good drug likeliness (**Table 3**). Most of the molecules exhibited physicochemical properties which fall in the range of known drugs as evidenced from # stars for compounds being 1 or 2 (QikProp. 2013). Molecules also lacked known toxicophores or reactive functional groups in all cases. The partition coefficient exhibited by QPlogPo/w were within the range 3.5–6.1. It was observed that most of the designed molecules exhibited QPPCaco within acceptable limits (**9a-j** and exhibited excellent value of QPPCaco). Human oral absorption of most of the compound is >80, which was considered to be good. Most of the designed molecules also followed the Lipinski's rule of five.

Conclusion

The present study revealed that 3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-amine derivatives possess good to moderate activity as antibacterial and antifungals. Out of 10 synthesized benzimidazole derivatives, compounds (9e,9h-j**)** possessed excellent activity (1.5-3.125 µg/mL). The promising activity of the 3-((1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazol-4-aminederivatives stablished them as crucial pharmacophore which can be used as lead to design further novel derivatives of benzimidazole with better antibacterial and antifungal activity. Considering this pharmacophore, the further expansion of the benzimidazole series are underway to find a potent antitubercular agent.The encouraging results from the antibacterial and antifungal studies impelled us to go for preliminary screening of synthesized molecules against *Mycobacteriumtuberculosis. Due to the better activity, some compound has been selected for further development and studies to acquire more reliable information about structure-activity relationship are in progress in our laboratory. Thus, these compounds could act as a good potential lead for further development of new antitubercular drugs.*Based on these literature reports, we found that benzimidazole is a unique as drug candidate scaffold in bacterial and fungal study. Moreover these observations indicate that the antibacerial activity depends not only on the triazole part but also on the functionalization of the benzimidazole derivatives. Importantly, substituents at the trazole ring attached to benzimidazole also play a crucial role in the biological activities. In summary, benzimidazole derivatives are regarded as potent antibacterial and antifungal agents. Surprisingly, in spite of their relevant anti-TB activities, little is still known about the mode of action and understanding of the implicated molecular mechanisms. We hope to learn more about these versatile molecules and its derivatives in addition to the synthesis of new, useful biologically important compounds in the near future.

Acknowledgments

Author is thankful to RCP kasegaon for Support for this work and ^{b}SureshGyanVihar University Mahal, Jagatpura, Jaipur.

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