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# Clubbed [1,2,3] triazoles by fluorine benzimidazole: A novel approach to H37Rv inhibitors as a potential treatment for tuberculosis

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ABSTRACT

A Novel Clubbed [1,2,3] triazoles with fluorine benzimidazole series of H37Rv strain inhibitors, potentially useful for the treatment of tuberculosis is disclosed on the basis of promising results of preliminary antimicrobial study. Evaluation of the SAR of substitution within these series has followed the identification of a range of compounds. Some of the derivatives are under further evaluation showing better considerable activity compared to rifampin.



Tuberculosis is primary cause of comparatively high mortality worldwide, despite the availability of highly active antitubercular drugs. The statistics shows that around three million people throughout the world die annually from tuberculosis[1,2] and today more people dies from tuberculosis than ever before.[3] Therefore, the development of new drugs with activity against multi drug-resistant (MDR) TB, extensively drug-resistant (XDR) TB, and latent TB is a priority task, which will shorten the current chemotherapy. As per the literature, after nitrogen, fluorine occupies the position of second favorite hetero-element in life science-oriented research. Over 10% of newly registered pharmaceutical drugs and some 40% of newly registered agrochemicals contain one or more fluorine atoms.[4] Fluorine containing benzimidazoles, which are showing biological activity, are well documented in the literature.[5] Along with this, the azoles antitubercular may be regarded as a new class providing truly effective drugs, which are reported to inhibit bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanisms.[6,7] Triazole, in particular, [1,2,3] triazole are among the various heterocycles that have broad pharmaceutical and medicinal applications like anti-HIV activity,[8] antimicrobial activity against gram positive bacteria, [9] inhibition of histidine biosynthesis, [10] ß-selective adrenergic receptor activity agonist, [11] bacterial and medicinal fungicides of second generation, [12] anti-inflammatory agents.[13] In addition to this, [1,2,3]-triazoles have found broad applications in agrochemicals as fungicides and plant growth regulator as well as industrial applications in dyes, corrosion inhibition (of copper and copper alloys) and photostabilizers.[14] In continuation, benzimidazole nucleus is an important pharmacophore in drug discovery having therapeutic applications.[15] So after extensive literature search, it was observed that, till date enough efforts have not been made to combine these two moieties as a single molecular scaffold and to study its antimicrobial activity followed by antitubercular activity against H37Rv strain. Along with this, literature reveals that, use of an electron withdrawing groups attached a benzimidazole ring, a heterocycle that, along with structure related benzthiazole is often found in molecules with antimycobacterial activity. In continuation of our research work to establish probable pharmacological activities of [1,2,3] triazole and fluorine benzimidazole, we herein report the synthesis of 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1-*H*-[1,2,3]-triazol-4-yl-methyl)-1*H*-benzo[*d*] imidazole **9a–9k** and its precursor and their inhibition of proliferation of H37Rv strain of M. Tuberculosis. In recent years, environmentally benign synthetic methods have received considerable attention. A fast, good yielding eco-friendly and catalyst-free chemical transformation by air oxidation **8**, followed by alkylation reaction for the synthesis of title compounds **9a–9k**, is designed.

Synthesis of 4-bromomethyl-1-(substituted-phenyl)-1H-[1,2,3]-triazole **5** is outlined in Scheme 1. To a solution of 2,3,4-triflu-

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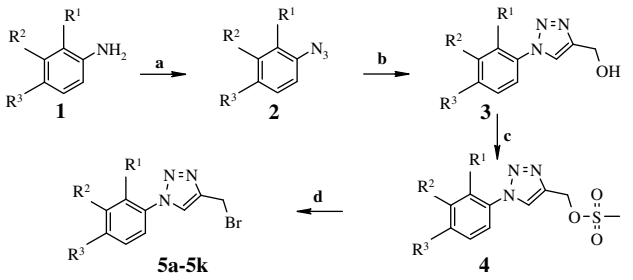


**Scheme 1.** (a) $NaNO_2$, HCl, $H_2O$, $NaN_3$; (b) propargyl alcohol, CuI, Acetonitrile; (c) Mesyl chloride, Triethylamine, dichloromethane; (d) LiBr, Acetone.

orobenzenamine **1a** (2.0 g, 13.6 mmol) dissolved in 50 mL HCl: $H_2O$ (1:1) was cooled at −5 °C by ice-salt mixture. Then a solution of sodium nitrite (1.87 g, 27.2 mmol) dissolved in water (15 mL) was added slowly at −5 °C. After completion of addition, the reaction mixture was stirred at −5 °C for 60 min. Then the reaction mixture was neutralized with sodium acetate (22.3 g, 272 mmol). Following this, a solution of $NaN_3$ (1.77 g, 27.2 mmol) in water (15 mL) was added slowly over the period of 30 min by maintaining the temp at −5 to 0 °C. After stirring for 30 min, the solution was allowed to warm at room temperature. Extracted with ethyl acetate (100 mL × 2), dried the organic layer over sodium sulphate and evaporated to yield 1-azido 2,3,4-trifluoro benzene **2a** as an oily product (1.8 g) (Scheme 2).

*In the second stage,* 1-azido 2,3,4-trifluoro benzene **2a** (1.8 g, 10.4 mmol) was dissolved in acetonitrile (25 mL). Propargyl alcohol (1.16 g, 20.8 mmol) and copper iodide (0.39 g, 5.2 mmol) were added to the above reaction mixture [16]. The reaction mixture was stirred at room temperature for 8–10 h, a solid material separated out, was filtered, suck dried. [1-(2,3,4-trifluorophenyl)-1*H*-1,2,3-triazol-4-yl] methanol **3a** (1.89 g) was obtained as off white solid.

*In the penultimate stage,* to a solution of [1-(2,3,4-trifluorophenyl)-1*H*-1,2,3-triazol-4-yl]-methanol (1.85 g, 8.07 mmol) dissolved in dichloromethane (20 mL) was added triethyl amine (1.22 g, 12.1 mmol) and mesyl chloride (1.1 g, 9.7 mmol) at room temperature. The reaction mixture stirred for 30 min and completion of reaction was monitored by TLC. Concentrate under vacuum and charged water, a solid material separates out, was filtered, suck dried. [1-(2,3,4-trifluorophenyl)-4-{methylsulphonyl}-methyl]-1*H*-1,2,3-triazole 4**a** (1.9 g) was obtained as off white solid.

*In the final stage,* to a solution of [1-(2,3,4-trifluorophenyl)-4-{methylsulphonyl}-methyl]-1*H*-1,2,3-triazole (1.9 g, 6.19 mmol) in acetone, charged lithium bromide (1.07 g, 12.37 mmol) and then refluxed for 1–2 h. After completion of reaction (monitored on TLC), distilled out acetone completely invacuo. Charged water, a solid material separates out, was filtered, suck dried. 4-(bromomethyl)-1-(2,3,4-trifluorophenyl)-1H-[1,2,3]-triazole (1.5 g) **5a** was obtained as buff colored solid.

Similarly, series of 4-(bromomethyl)-1-substituted-phenyl-1H-[1,2,3]-triazole **5a–5k** was synthesized by using the respective amine of **1a–1k**. All the compounds were characterized by $^1$H NMR and MS.

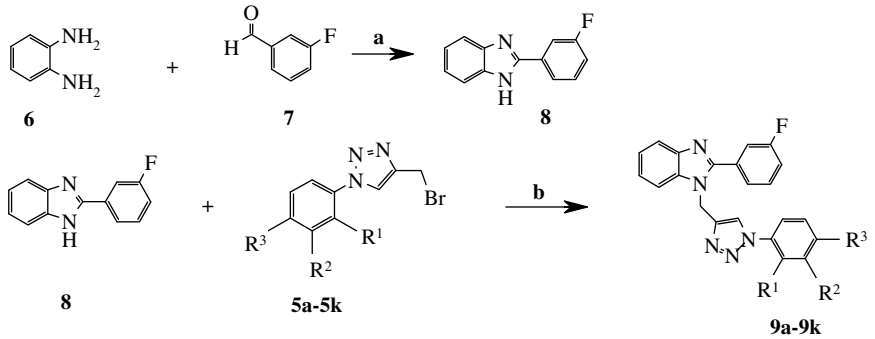
We have synthesized the target molecule, 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1-*H*-[1,2,3]-triazol-4-yl-methyl)-1*H*-benzo[*d*] imidazole **9a–9k**. Condensation of *o*-phenylenediamine **6** with 3-fluoro benzaldehyde **7** in toluene or xylene at 110 °C for 1 h by air oxidation without using oxidizing agents or catalysts, furnished the 2-(3-fluoro-phenyl)-1H benz [*d*] imidazole **8**. The synthesized intermediate was characterized by $^1$H NMR and MS. Yield-73%; MS: *m*/*z* 213.2 ($M^+$); $^1$H NMR ($CDCl_3$, 400 MHz), $\delta$ (ppm): 7.4 (m, 2H, ArH), 7.21 (m, 2H, ArH), 7.12–7.16 (m, 2H, ArH), 5.65 (s, 1H, $-CH_2$).

Then the subsequent reaction of 3-fluoro benzimidazole **8** with substituted 4-(bromomethyl)-1-phenyl-1H-[1,2,3]-triazole **5a–5k** using NaH as a base proceeded at room temperature to furnish 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1H-[1,2,3]-triazol-4-yl methyl)-1*H*-benzo[*d*]imidazole derivatives **9a–9k**. All the compounds were characterized by $^1$H NMR, FTIR, MS and elemental analysis.[17–20]

The in vitro antimicrobial activity of **9a–9k** were assessed against two representatives of Gram-positive and Gram-negative bacteria viz. *Staphylococcus aureus, Pseudomonas* aeruginosa, Escherichia coli and Salmonella typhosa. From the antibacterial screening it was observed that all compounds exhibited activity against different organisms employed. Looking at the structure activity relationship, marked inhibition in bacteria was observed in the compounds **9a, 9b, 9c, 9g** and **9h**, whereas **9d, 9e, 9f, 9i, 9j**, and **9k** have shown moderate to least activity. Our results revealed that, maximum compounds, which are showing better antimicrobial activities with reference to Gentamycin, had 'Fluorine'. This indicates that 'Fluorine' may be playing an important role in the activity of the compounds. The encouraging results (Table 1) from the antibacterial activity prompted us to opt for preliminary screening of the titled compounds for their antitubercular activity. The results of the in vitro evaluation of antimycobacterial activity are reported in Tables 2 and 3.

At the commencement of this program, compound **9a** was the only target, which had 'F'. As our program progressed, it have been observed that this target with fluoro substitution possess enhanced anti-mycobacterial activity, >96% of inhibition at 6.25 mg concentration while other compounds **9d, 9e, 9h, 9j** and **9k** exhibited less than 90% inhibition at the same concentration.

Thus, we have considered **9a** as a lead molecule and subsequent structural modifications were carried out using several alternatives for fluoro substitution. As a first step towards lead optimization, we have incorporated 'F' at 2, 3 & 4 in different variations, wherein



**Scheme 2.** (a) Toluene, 110 °C, 30–60 min.; (b) NaH, 4-bromomethyl-1-phenyl-1H-[1,2,3]-triazole, DMF, rt.

**Table 1**
Antibacterial activity[21] of the compounds **9a–9k** (The value indicates bacterial growth inhibition measured in mm).

| Compound | Organisms | | | |
|---|---|---|---|---|
| | Sa | Pa | Ec | St |
| **9a** | 31 | 33 | 29 | 30 |
| **9b** | 32 | 31 | 30 | 29 |
| **9c** | 26 | 19 | 20 | 19 |
| **9d** | 21 | 24 | 19 | 23 |
| **9e** | 20 | 26 | 18 | 21 |
| **9f** | 18 | 13 | 21 | 18 |
| **Gent** | 34 | 35 | 31 | 30 |
| **9g** | 33 | 34 | 32 | 31 |
| **9h** | 26 | 28 | 17 | 18 |
| **9i** | 18 | 13 | 21 | 18 |
| **9j** | 16 | 19 | 14 | 17 |
| **9k** | 18 | 20 | 19 | 17 |
| **Gent** | 34 | 35 | 31 | 30 |

Sa: *Staphylococcus aureus*, Ec: *Escherichia coli*, Pa: *Pseudomonas aeruginosa*, St: *Salmonella typhosa*, Gent: Gentamycin.

**Table 2**
First antituberculosis screening of compounds **9a–9k**.

| Compound | $R^1$ | $R^2$ | $R^3$ | MIC[a] | GI (%)[b] |
|---|---|---|---|---|---|
| **9a** | F | F | F | <6.25 | — |
| **9b** | H | F | F | <6.25 | 96 |
| **9c** | H | F | H | <6.25 | 96 |
| **9d** | OMe | H | H | <6.25 | — |
| **9e** | H | H | Ome | <6.25 | 96 |
| **9f** | F | H | Me | <6.25 | 96 |
| **9g** | F | H | F | <6.25 | 96 |
| **9h** | H | H | H | <6.25 | — |
| **9i** | H | H | $CF_3$ | <6.25 | — |
| **9j** | Me | H | H | <12.5 | — |
| **9k** | H | H | Me | <12.5 | — |

[a] MIC in ($\mu g/mL^{-1}$). MIC of rifampin: 0.015–0.125 mg $mL^{-1}$ versus M. tuberculosis H37Rv (97% inhibition).
[b] Growth inhibition of virulent H37Rv strain of M. tuberculosis.

**Table 3**
Second level antituberculosis screening

| SN | MIC ($\mu M$)[a] |
|---|---|
| **9b** | 0.34 |
| **9c** | 0.58 |
| **9e** | 6.25 |
| **9f** | 3.13 |
| **9g** | 0.32 |

[a] Actual minimum inhibitory concentration (MABA assay).

all the alterations made **9b, 9c, 9f** and **9g**, shown promising activity, that is, >96% of inhibition at 6.25 mg concentration, proving that the modifications are towards synthesis of a pharmacophore. The next structural modification done was a trifluoromethyl substitution product, **9i** but this change resulted in a substantial loss of biological activity. This loss may indicate retardation in the intracellular transport due to highly electronegativity in one region. In case of electron donating groups, like methyl substitutions resulted in loss of activity. The biological data generated reveals that compounds having an electron-withdrawing group attached may prove a template for anti tuberculosis activity for further development. It was also observed that the promising antimicrobials have proved to be better antimycobacterials. Results obtained clearly related to the electron withdrawing ability of the substituents on the benzyl nucleus with heterocyclic ring. Even though cytotoxicity has not been evaluated for this series of molecules. So compound could form a promising core for lead optimization.

All the compounds, **9b, 9c, 9e, 9f** and **9g** that were active in the first level screening were then tested to determine the actual minimum inhibitory concentration (MIC), wherein, compounds **9b, 9c** and **9g** have been proven to be the most active, with MIC values ranging from 0.32 to 0.58.

In conclusion, the antimycobacterial screening of the novel series has demonstrated emergence of potent derivatives that has highly electronegative part, that is, 'Fluorine' and may be due to the presence of [1,2,3]-triazole ring attached to the benzimidazole. Specifically compounds **9b, 9c** and **9g**, due to the better activity against the mycobacteria, are the best choice for the preparations of new derivatives in order to improve its effectiveness on intracellular mycobacteria (macrophage) or in infected animal. Also, we have developed an efficient methodology for the synthesis of a 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1H-[1,2,3]-triazol-4-yl-methyl)-1*H*-benzo[*d*] imidazole derivatives and towards the development of new pharmacophore. Finally it can be concluded that an ideal antimycobacterial agent with minimal toxicity and potential activity can be designed using above said compounds as lead molecules.

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17. *Experimental*: The melting points were estimated by Veggo programmable (microprocessor based) melting point apparatus and are uncorrected. 1H NMR spectra were recorded on a Varian 400 MHz spectrometer MHz instrument using $CDCl_3$ as solvent using TMS as internal standard; the chemical shifts (δ) are reported in ppm Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), ds (double singlet), dd (double doublet), m (multiplet) and br s (broad singlet). IR spectra were recorded on (KBr disc) using a FTIR bruker Vector 22 Spectrophotometer. Elemental analyses were determined on Elementor Vario instrument. EI-MS spectra recorded on micromass-quatro – II. The purity of the compounds was checked on Merck precoated silica gel 60 F-254. All the reagents, solvents used were of commercial grade only.
18. General experimental procedure for the synthesis of 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1H-[1,2,3]-triazol-4-yl-methyl)-1*H*-benzo[*d*] imidazole **9a–9k**. To a suspension of sodium hydride (0.94 mmol) in dimethyl formamide was added 2-(3-fluorophenyl)-1H-benz[*d*] imidazole (8) (0.47 mmol) at room temperature and stirred the reaction mixture for 30 min. To this reaction mixture, substituted 4-bromomethyl-1H-[1,2,3]-triazole (5a–i) (0.52 mmol) was then added at room temperature and stirred for 30 min. After completion, the reaction mixture was quenched with ice water. The solid that separates was filtered and dried at room temperature **9a–9k**.
19. Synthesis of 2-(3-fluoro-phenyl)-1[1-(2,3,4-trifluoro-phenyl)-1H-[1,2,3]-triazol-4-yl-methyl]-1H-benz [*d*] imidazole (**9a**). The compound was obtained using 4-bromomethyl-1-(2,3,4-trifluorol-phenyl)-4H-[1,2,3]-triazole (5a) as a buff colour crystalline solid (**9a**). Yield 74%; mp: 178-180 °C; IR (KBr): 3064, 1591, 1521, 1046, 748 $cm^{-1}$.; $^1H$ NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.86 (s, 1H, CH-triazole ring), 7.82 (d, 1H, ArH), 7.67 (m, 3H, ArH), 7.51 (m, 2H, ArH), 7.30 (dd, 2H, ArH), 7.26 (m, 1H, ArH), 7.15 (dd, 1H ArH,), 5.65 (s, 2H, $CH_2$); MS: *m*/*z* 424.2 ($M^+$); Anal. calcd for $C_{22}H_{13}F_4N_5$: C, 62.41; H, 3.10; N, 16.54; found C, 62.43; H, 3.18; N, 16.66.
20. By the procedure mentioned in the reference [19], we have synthesized all the compounds **9a**–**9k** and characterized by $^1H$ NMR, FTIR, MS and elemental analysis.
21. *Antimicrobial activity*: The title compounds were screened for the antimicrobial activity against different microorganisms under the following conditions. (Table 3) Method: Well diffusion method[22], Medium: The nutrient agar medium, solvent: chloroform: concentrations: 50 and 100 μM. Condition: 24 h at 24–28 °C, Standard: The antibiotic Gentamycin The nutrient agar medium, 20 mL was poured into the sterile petri dishes. To the solidified plates, wells were made using a sterile cork borer 10 mm in diameter. The 24 h sub-cultured bacteria was inoculated in the petri-plates, with a sterile cotton swab dipped in the nutrient broth medium. After inoculating, the compounds were dissolved separately with the chloroform solvent and poured into the wells with varying concentrations ranging from 50 and 100 μM using a micropipette. The plates were left over for 24 h at 24–28 °C. The antibiotic Gentamycin was used as a standard for comparative study. The percentage of inhibition was calculated by the formula % Inhibition = Diameter of the inhibition zone × 100.
22. *Antitubercular activity*: Primary screening was conducted at 6.25 μg $mL^{-1}$ against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). [23] Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system. [24,25] Compounds showing more than/95% inhibition in the primary screening were considered active and then re-tested at a lower concentrations against *M. tuberculosis* H37Rv in order to determine the actual MIC, using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 95% with respect to the controls. Rifampin (RMP) was used as the reference compound (RMP MIC=/0.015–0.125 mg $mL^{-1}$). We also have done cytotoxicity analysis of the above-synthesized compounds, using neutral red uptake by using Vero-C-1008 cell line at various concentrations (6.25–50 μg/mL), none of them were found toxic. Hence the activities of the above-synthesized compounds were not due to cytotoxicity of compounds.
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