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Original article

SAR study of clubbed [1,2,4]-triazolyl with fluorobenzimidazoles as antimicrobial and antituberculosis agents

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ABSTRACT

In the present study, a series of novel 2-[4-(1*H*-[1,2,4]-triazol-1-yl)phenyl]-1-substituted-4,6-difluoro-1*H*-benzo[*d*]imidazole derivatives are synthesized by the alkylation of 2-[4-(1*H*-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1*H*-benzo[*d*]imidazole with substituted alkyl and aryl halides. The compounds were evaluated for their preliminary *in-vitro* antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhosa* and then were screened for their antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain by broth microdilution assay method. The antibacterial data suggested that the analogs with electronegative substituents emerged as promising antimicrobials. It was also observed that the promising antimicrobials have proved to be better antimycobacterials. Few of selected analogs are under further evaluation for secondary antitubercular screening, as they have shown better activity compared to rifampin.

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1. Introduction

Despite the availability of highly potential antitubercular agents, tuberculosis remains primary cause of comparatively high mortality worldwide. The statistics shows that around three million people throughout the world die annually from tuberculosis [1,2] and today more people die 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. Although new agents that will shorten the duration of current chemotherapy are also needed. A special interest has been focused on five membered heterocyclic compounds like imidazole, pyrrole, oxadiazole, specially triazoles and other heterocyclic system derivatives have been reported [4]. The azole antitubercular may be regarded as a new class providing truly effective drugs, which is reported to inhibit bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanisms [5,6].

Heterocyclic species like triazole and fluorobenzimidazole derivatives [7] represent a novel emerging major chemical entity as antimicrobial agent. Triazoles, in particular, [1,2,4]-triazole counterparts of fluorobenzimidazoles, are among the various heterocycles that have received much attention as potential antimicrobial agents, antifungal, anti-inflammatory, CNS stimulants, sedatives, antianxiety [8,9]. Benzimidazole moiety has already been reported for its antimicrobial activity [10,11] along with antiviral, antiulcer, antihypertension [12], cardiotonic [13] Literature survey shows that 2-substituted benzimidazoles are found to be more potent and hence the design and synthesis of 2-substituted benzimidazole are the potential area of research [14]. 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 biological activity. In continuation with our earlier work [15] to establish probable pharmacological activities of [1,2,4]-triazoles [16–22] and benzimidazole [23], we herein report the synthesis of new [1,2,4]-triazole derivatives clubbed with fluorobenzimidazole moiety.

In recent years, environmentally benign synthetic methods have received considerable attention [24–33]. A fast, highly efficient and eco-friendly, catalyst-free, chemical transformation by air

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oxidation, for the synthesis of title compounds is designed. We now wish to disclose our results towards the synthesis of antibacterial and antimycobacterial activity of 2-[4-(1*H*-[1,2,4]-triazol-1-yl)phenyl]-1-substituted-4,6-difluoro-1*H*-benzo[*d*]imidazole derivatives (**9**) Schemes 1–3.

2. Results and discussion

2.1. Chemistry

The synthesis of title compounds was started with condensation of 3,5-difluorobenzene-1,2-diamine (**8**) [35] condensed with 4-(1*H*-[1,2,4]-triazol-1-yl) benzaldehyde (**3**) [34] in refluxing toluene for 1 h by air oxidation without using any oxidizing agent or catalyst, to furnish 2-[4-(1*H*-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1*H*-benzo[*d*]imidazole (**9**). The chemical transformation of (**9**) with alkyl and substituted aryl halides was carried out using NaH as a base proceeded at room temperature to furnish 2-[4-(1*H*-[1,2,4]-triazol-1-yl)phenyl]-1-substituted-4,6-difluoro-1*H*-benzo[*d*]imidazole derivatives. The physical and spectral data of compounds (**10a**–**10o**) are presented in Section 3.3.

2.2. Structure–activity relationship study

In an on-going research work, we have synthesized benzimidazoles clubbed with different heteroaromatic nucleus like [1,2,3]-triazole, [1,2,4]-oxadiazole, [1,2,4]-triazole etc. These newly synthesized scaffolds were screened for antimicrobial activities against various gram +ve and gram −ve microorganisms. Compound 2-[4-(1*H*-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1*H*-benzo[*d*]imidazole (**9**) exhibited better antimicrobial activity as compared to other scaffolds.

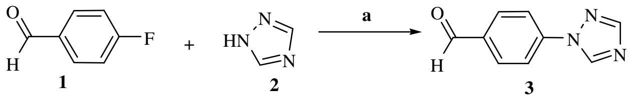
Having confirmed the activity of compound (**9**), we embarked on a hit-to-lead exploration program focusing on N^1–H of imidazole nucleus. The NH was alkylated with different alkyl and substituted aryl halides. Thus our aim was to explore SAR trends and to find out the lead for further optimization.

2.2.1. Antimicrobial activity

The newly synthesized compounds (**10a**–**10o**) were evaluated for *in-vitro* antimicrobial activities. They were assessed against two representative of each gram +ve and gram −ve strains like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhosa*. Gentamycin was used as a reference drug molecule. The data generated from preliminary screening showed that compounds displayed moderate to better activity. SAR study reveals that compounds like **10a**, **10e**, **10f**, **10h**, **10i**, and **10l** displayed maximum activity, while compound **10n** was moderately active. The other compounds viz. **10b**, **10c**, **10d**, **10g**, **10j**, **10k**, **10m**, and **10o** showed least activity. This *in-vitro* antimicrobial activity data are tabulated in Table 3.

2.2.2. Antitubercular activity

The encouraging results from the antibacterial activity prompted us to opt for preliminary screening of the titled compounds for their antitubercular activity. The antitubercular activity data are mentioned in Tables 1 and 2



a: K_2CO_3, DMF, 120°C

Scheme 1.

At the commencement of this study in the preliminary screening, compound (**9**) displayed better activity and showed >95% inhibition at 6.25 mg concentration. This result encouraged us to consider compound (**9**) as our lead molecule. Subsequent structural modifications were carried out for lead optimization.

The first step towards lead optimization was incorporation of simple alkyl groups like CH_3, CH_2CH_3, and $CH_2CH_2CH_3$ at NH of imidazole ring system. The antituberculosis study data of these compounds showed <90% of inhibition at 6.25 mg concentration. The next structural modification was introduction of more electronegative groups viz. $CH_2CH{=}CH_2$, CH_2CCH, CH_2CN, and $CH_2C_6H_5$. This change resulted in substantial improvement in activity. More than 96% inhibition at 6.25 mg concentration was noticed. Enhancement in activity proves that this modification is step up towards synthesis of a pharmacophore. On the basis of biological data, improvement in biological activity was observed with increase in electronegativity of molecule. Hence we planned to introduce electron withdrawing and donating groups at different positions to study its influence on activity. Different analogs with electron withdrawing groups e.g. fluorine and chlorine and donating groups like methyl and methoxy were synthesized. These analogs were evaluated for antimicrobial activity. In majority of the cases, compounds having electronegative atoms like fluorine and chlorines (**10h**, **10i** and **10l**) exhibited good activity i.e. >96% inhibition at 6.25 mg concentration. In exceptional case, 3-methoxy substituted analog was also found better in activity. The same result was applicable only in primary screening while in secondary level screening same response was not produced. Therefore we have introduced trifluoromethyl substitution (**10k**) to increase electronegativity. This change resulted in substantial loss in activity. This loss in activity may be due to retardation in intracellular transport because of high electronegativity.

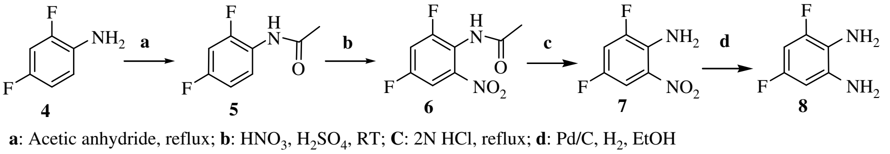
All those compounds which were found to be active in first level of screening viz. **10e**, **10f**, **10h**, **10i** **10l** and **10n** then tested to determine actual minimum inhibitory concentration. We have drawn the inference that compounds **10h** and **10i** have proved to be the best and most active with MIC value ranging from 0.36 to 0.58.

2.2.3. Conclusion

A series of novel analogs of fluorobenzimidazole clubbed with [1,2,4]-triazoles were synthesized and assessed for their antibacterial and antituberculosis H37Rv strain activity. The antibacterial data indicates that the analogs with electronegative substituents emerged as promising antimicrobials showing better to moderate activity. It was also observed that the promising antimicrobials have proved to be better antimycobacterials. Specially, compounds **10h** and **10i**, due to their better activity against the H37Rv strain, are the best choice for the preparation of new derivatives in order to improve antimycobacterial activity in future. Also, we have developed an efficient methodology for the synthesis of **10h** and **10i** benzimidazole derivatives towards the development of new pharmacophore. Such activity and their ease of preparation make them attractive candidate for further assessment in antituberculosis agents.

3. Experimental section

The melting points were estimated by Veggo programmable (microprocessor based) melting point apparatus and are uncorrected. ^{1}H 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),



a: Acetic anhydride, reflux; **b**: HNO_3, H_2SO_4, RT; **C**: 2N HCl, reflux; **d**: Pd/C, H_2, EtOH

Scheme 2.

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.

*3.1. General experimental procedure for the synthesis of 4-(1*H*-[1,2,4]-triazol-1-yl)benzaldehyde (**3**)*

To a solution of 4-fluorobenzaldehyde (**1**) (5.0 g, 40.32 mmol) and 1*H*-[1,2,4]-triazole (**2**) (3.3 g, 47.82 mmol) in DMF (50 mL) was added powdered K_2CO_3 (6.67 g, 48.38 mmol). The mixture was stirred at 120 °C for 12 h. Reaction mixture was warmed up at room temperature and quenched with water. It was extracted with ethylacetate (2 × 150 mL). The combined organic fractions were washed with water (100 mL) followed to brine solution (100 mL) and dried over anhydrous Na_2SO_4. Solvent was removed under reduced pressure and purification was done by flash chromatography (hexane:ethylacetate, 3:1 elution) to give 4-(1*H*-[1,2,4]-triazol-1-yl)benzaldehyde as a off-white solid (**3**) (5.0 g, 72% yield). ^1H NMR ($CDCl_3$) δ (ppm): 8.01–8.10 (4H, m), 8.15 (1H, s, triazole-H), 9.43 (1H, s, ArH), 10.10 (1H, s, CHO). *m*/*z* 173 (M^+).

*3.2. General experimental procedure for the synthesis of 3,5-difluorobenzene-1,2-diamine (**8**)*

Step I: acetic anhydride (7.9 g, 77.5 mmol) was added slowly to 2,4-difluorobenzenamine (**4**) (10 g, 77.5 mmol) at 0–5 °C, where upon a solid material precipitates out after 15–20 min. Reaction mixture was quenched in ice water and stirred for 30 min. A off-violet colour solid material separates out, filtered and suck dried to obtained as *N*-(2,4-difluorophenyl)acetamide. (**5**) (12.0 g, 91% yield). *m*/*z* 172 (M).

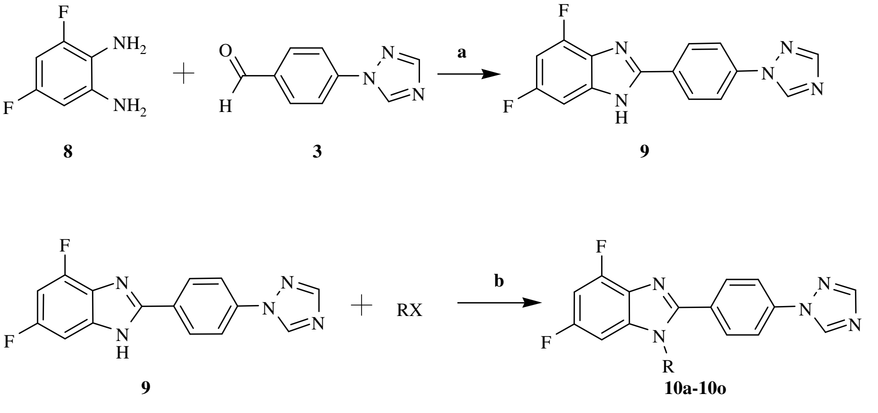
Step II: nitration of *N*-(2,4-difluorophenyl)acetamide (**5**) was carried out (12 g, 70.2 mmol) in nitric acid (25 mL), by the addition of conc. sulfuric acid (25 mL) slowly at 5–10 °C. Then gradually warming up the reaction mixture at room temperature and stirring was continued for 2–3 h. Monitored the progress of the reaction by TLC system (ethylacetate:hexane, 2:8). Quenched the reaction in ice water, a pale yellow colour solid precipitates out, filtered and suck dried to give as *N*-(2,4-difluoro-6-nitrophenyl)acetamide (**6**) (12.0 g, 80% yield). ^1H NMR ($CDCl_3$) δ (ppm): 2.23 (3H, s, CH_3), 7.26 (1H, m, ArH), 7.62 (1H, m, ArH), 8.01 (1H, s, –NH). *m*/*z* 215 $(M)^-$.

Step III: *N*-(2,4-difluoro-6-nitrophenyl)acetamide (**6**) (12 g, 55.5 mmol) was suspended in 2 N HCl (50 mL) and refluxed for 3–4 h. The reaction mixture was cooled gradually to room temperature, neutralized with aq. $NaHCO_3$ solution and where upon a yellow colour needle type crystal separates out. It was filtered, suck dried to give as 2,4-difluoro-6-nitrobenzenamine. (**7**) (7.0 g, 73% yield). ^1H NMR ($CDCl_3$) δ (ppm): 6.05 (2H, s, NH_2), 7.12 (1H, m, ArH), 7.73 (1H, s, ArH). *m*/*z* 173 $(M)^-$.

Step IV: 2,4-difluoro-6-nitrobenzenamine. (**7**) (7.0 g, 40.2 mmol) was hydrogenated in ethanol solution by using 10% Pd/C (700 mg) under 60 psi H_2 pressure. Monitored the progress of the reaction on TLC system (ethyl acetate:hexane, 1:1). After completion, catalyst was filtered. Solvent was removed under reduced pressure The final product 3,5-difluorobenzene-1,2-diamine (**8**) was purified by sublimation under vacuum, a cotton like off-white material obtained (4.9 g, 85% yield).

*3.3. General experimental procedure for the synthesis of 2-[4-(1*H*-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1*H*-benzo[*d*]imidazole (**9**)*

A mixture of 4-(1*H*-[1,2,4]-triazol-1-yl)benzaldehyde (**3**) (5.0 g, 28.9 mmol) and 3,5-difluorobenzene-1,2-diamine (**8**) (6.0 g, 41.7 mmol) in toluene (25 mL) was refluxed for 2–3 h. The reaction mixture was then cooled to 5 °C gradually, stirred for 15–20 min. The pale yellow solid material formed was filtered through buchner funnel and washed with ice-cold toluene. The obtained pale yellow



a: 4-(1*H*- [1,2,4]-triazol-1-yl)benzaldehyde, toluene,110 °C; **b**: NaH, RX, DMF, RT

Scheme 3.

Table 1
First antituberculosis activity of compounds **10a**–**10o**.

Compound	R	MIC[a]	GI (%)[b]
10a	$-CH_3$	<6.25	–
10b	$-CH_2CH_3$	<6.25	–
10c	$-CH_2CH_2CH_3$	<6.25	–
10d	$-CH_2CH{=}CH_2$	<6.25	–
10e	$-CH_2CCH$	<6.25	96
10f	$-CH_2CN$	<6.25	96
10g	$-CH_2C_6H_5$	<6.25	–
10h	$-CH_2(3\text{-}FC_6H_5)$	<6.25	96
10i	$-CH_2(3\text{-}ClC_6H_5)$	<6.25	96
10j	$-SO_2C_6H_5CH_3$	<6.25	–
10k	$-CH_2(3\text{-}CF_3C_6H_5)$	<6.25	–
10l	$-CH_2(4\text{-}FC_6H_5)$	<6.25	96
10m	$-CH_2(3\text{-}MeC_6H_5)$	<6.25	–
10n	$-CH_2(3\text{-}OMe\ C_6H_5)$	<6.25	96
10o	$-CH_2(2\text{-}MeC_6H_5)$	<6.25	–
Rifampin	–	0.015–0.125	97

[a] MIC in 1μg/ml^{-1}.

[b] Growth inhibition of virulent H37Rv strain of *M. tuberculosis*.

colour solid (**9**) was triturated with diethyl ether and filtered (7.5 g, 61% yield), m.p. 220–222 °C. ^{1}H NMR ($CDCl_3$, 400 MHz), δ (ppm): 7.07 (1H, m, ArH), 7.26 (1H, m, ArH), 8.07 (2H, d, $J = 8.17$ Hz, ArH), 8.27 (1H, s, triazole-H), 8.32 (2H, d, $J = 8.21$ Hz, ArH), 9.39 (1H, s, triazole-H) 12.18 (1H, s, NH). *m*/*z* 298.1 (M). Anal. calcd for $C_{15}H_9F_2N_5$: C, 60.61; H, 3.05; N, 23.56; found C, 60.75; H, 3.10; N, 23.64.

3.4. General experimental procedure for the 2-[4-(1H-[1,2,4]-triazol-1-yl) phenyl]-1-substituted-4,6-difluoro-1H-benzo[d] imidazole derivatives (**10a**–**10o**)

To a solution of sodium hydride (1.1 eq.) in dimethyl formamide was added 2-[4-(1*H*-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1*H*-benzo[*d*]imidazole (**9**) (1.0 eq.), stirred the reaction mixture at room temperature for 30 min. To this reaction mixture, charge substituted aryl or alkyl halide (1.2 eq.) and continue stirring for 30 min. After completion of reaction, the reaction mixture was quenched in the ice water, a solid precipitates out, was filtered, with bed wash of water (100 mL) and suck dried (yield = 69–81%).

3.4.1. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1-methyl-1H-benzo[d]imidazole (**10a**)

The compound obtained as a pale green colour crystalline solid by using iodomethane (yield 72%), m.p. 100–102 °C. IR (KBr, cm^{-1}): 3328, 3104, 1602, 1502, 1279, 1214, 1149, 980, 838, 672. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 3.81 (3H, s, CH_3), 7.13 (1H, m, ArH), 7.29 (1H, m, ArH), 8.14 (2H, d, $J = 8.06$ Hz, ArH), 8.26 (1H, s, triazole-H), 8.28 (2H, d, $J = 8.08$ Hz, ArH), 9.29 (1H, s, triazole-H), *m*/*z*: 312.2 (M$^+$). Anal. calcd. for $C_{16}H_{11}F_2N_5$: C, 61.73; H, 3.56; N, 22.50; found C, 61.74; H, 3.56; N, 22.55.

Table 2
Second level antituberculosis screening.

SN	MIC[a] μg/ml^{-1}
10e	3.13
10f	1.56
10h	0.36
10i	0.58
10l	3.13
10n	3.13
Rifampin	0.015–0.125

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

Table 3
First antibacterial screening of compounds **10a**–**10o**.

Compound	R	Organisms			
		Sa	Pa	Ec	St
10a	$-CH_3$	28	26	21	19
10b	$-CH_2CH_3$	23	18	16	14
10c	$-CH_2CH_2CH_3$	21	23	18	19
10d	$-CH_2CH{=}CH_2$	20	22	23	23
10e	$-CH_2CCH$	25	23	21	24
10f	$-CH_2CN$	27	26	24	20
10g	$-CH_2C_6H_5$	19	20	15	13
10h	$-CH_2(3\text{-}FC_6H_5)$	29	26	22	24
10i	$-CH_2(3\text{-}ClC_6H_5)$	26	22	19	18
10j	$-SO_2C_6H_5CH_3$	14	12	16	16
10k	$-CH_2(3\text{-}CF_3C_6H_5)$	22	21	20	18
10l	$-CH_2(4\text{-}FC_6H_5)$	25	23	19	21
10m	$-CH_2(3\text{-}MeC_6H_5)$	21	18	18	16
10n	$-CH_2(3\text{-}OMe\ C_6H_5)$	24	22	22	21
10o	$-CH_2(2\text{-}MeC_6H_5)$	19	21	18	14
Gentamycin	–	34	35	31	30

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

3.4.2. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-1-ethyl 4,6-difluoro-1H-benzo[d]imidazole (**10b**)

The compound obtained as a pale yellow colour crystalline solid by using iodoethane (yield 78%), m.p. 148–150 °C. IR (KBr cm^{-1}): 3334, 3099, 1636, 1521, 1435, 1348, 1279, 1216, 1101, 981, 842, 673. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 1.46 (3H, t, CH_3), 4.28 (2H, q, CH_2), 7.06 (1H, m, ArH), 7.27 (1H, m, ArH), 7.80 (2H, d, $J = 7.92$ Hz, ArH), 8.07 (2H, d, $J = 7.94$ Hz, ArH), 8.52 (1H, s, triazole-H), 9.18 (1H, s, triazole-H), *m*/*z*: (M$^+$) 326.2; Anal. calcd. for $C_{17}H_{13}F_2N_5$: C, 62.76; H, 4.03; N, 21.53; found C, 62.77; H, 4.27; N, 21.55.

3.4.3. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1-propyl-1H-benzo[d]imidazole (**10c**)

The compound obtained as a off-white colour crystalline solid by using 1-bromopropane (yield 79%), m.p. 138–140 °C. IR (KBr cm^{-1}): 3091, 2985, 1639, 1539, 1413, 1280, 1110, 981, 846, 669. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.71 (3H, t, CH_3), 1.69 (2H, m, CH_2), 4.29 (2H, q, CH_2), 7.14 (1H, m, ArH), 7.41 (1H, m, ArH), 7.94 (2H, d, $J = 8.0$ Hz, ArH), 8.10 (1H, s, triazole-H), 8.29 (2H, d, $J = 8.01$ Hz, ArH), 9.40 (1H, s, triazole-H), *m*/*z*: (M$^+$) 340.1. Anal. calcd. for $C_{18}H_{15}F_2N_5$: C, 63.71; H, 4.46; N, 20.64; found C, 63.76; H, 4.55; N, 20.79.

3.4.4. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-1-allyl-4,6-difluoro-1H-benzo[d]imidazole (**10d**)

The compound obtained as a yellow colour crystalline solid by using 3-bromoprop-1-ene (yield 68%). m.p. 156–158 °C. IR (KBr cm^{-1}): 3263, 3129, 1640, 1539, 1436, 1366, 1275, 982, 844, 796, 674. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 4.18 (2H, s, CH_2), 6.76 (2H, m, ArH), 6.71 (1H, m, allylic), 7.48 (1H, m, ArH), 7.66 (1H, m, ArH), 7.81 (2H, d, $J = 7.92$ Hz, ArH), 8.08 (2H, d, $J = 7.95$ Hz, ArH), 8.14(1H, s, triazole-H), 8.65 (1H, s, triazole-H), *m*/*z*: (M$^+$) 338.1. Anal. calcd. $C_{18}H_{13}F_2N_5$ for: C, 64.09; H, 3.88; N, 20.76; found C, 64.10; H, 3.91; N, 20.81.

3.4.5. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1-(prop-2-ynyl)-1H-benzo[d]imidazole (**10e**)

The compound obtained as a buff colour crystalline solid by using 3-bromoprop-1-yne (yield 70%). m.p. 180–182 °C. IR (KBr cm^{-1}): 3193, 3125, 1690, 1599, 1505, 1430, 1388, 1275, 982, 844, 674. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 3.58 (1H, s, prop-2-ynyl-*H*), 5.22 (2H, d, CH_2), 7.21 (1H, m, ArH), 7.48 (1H, m, ArH), 8.06 (2H, d, $J = 8.07$ Hz, ArH), 8.15 (2H, d, $J = 8.08$ Hz, ArH), 8.30 (1H, s, triazole-H), 9.43 (1H, s, triazole-H), *m*/*z*: (M$^+$) 336.1. Anal. calcd. for

$C_{18}H_{11}F_2N_5$: C, 64.48; H, 3.31; N, 20.89; found C, 64.49; H, 3.41; N, 20.98.

*3.4.6. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1H-benzo [d]imidazole-1-acetonitrile (**10f**)*

The compound obtained as a buff colour crystalline solid by using bromoacetonitrile (yield 72%). m.p. 216–218 °C. IR (KBr cm^{-1}): 3112, 2932, 1641, 1604, 1503, 1431, 1226, 1122, 981, 845, 674. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.42 (2H, s, CH_2), 7.23 (1H, t, ArH), 7.48 (1H, dd, ArH), 8.09 (2H, d, $J = 7.98$ Hz, ArH), 8.21 (2H, d, $J = 7.97$ Hz, ArH), 8.41 (1H, s, triazole-H), 9.45 (1H, s, triazole-H), *m/z*: 337.2 (M$^+$); Anal. calcd. for $C_{17}H_{10}F_2N_6$: C, 59.63; H, 2.50; N, 26.08; found C, 59.81; H, 2.71; N, 25.99.

*3.4.7. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-1-benzyl-4,6-difluoro-1H-benzo[d]imidazole (**10g**)*

The compound obtained as a off-white colour crystalline solid by using 1-(bromomethyl)benzene (yield 81%). m.p. 172–174 °C. IR (KBr cm^{-1}): 3113, 2361, 1643, 1597, 1502, 1455, 1358, 1173, 1078, 982, 847, 724, 672. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 5.62 (2H, s, CH_2), 6.96 (2H, d, ArH), 7.12 (2H, m, ArH), 7.25–7.32 (3H, m, ArH), 7.95 (2H, d, $J = 7.97$ Hz, ArH), 8.05 (2H, d, $J = 7.98$ Hz, ArH), 8.27 (1H, s, triazole-H), 9.37 (1H, s, triazole-H), *m/z*: 388.2 (M$^+$). Anal. calcd. for $C_{22}H_{15}F_2N_5$: C, 68.21; H, 3.90; N, 18.08; found C, 68.23; H, 3.92; N, 18.11.

*3.4.8. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-1-(3-fluorobenzyl)-4,6-difluoro-1H-benzo[d]imidazole (**10h**)*

The compound obtained as a off-white colour crystalline solid by using 1-(bromomethyl)-3-fluorobenzene (yield 74%). m.p. 148–152 °C. IR (KBr cm^{-1}): 3133, 2359, 1651, 1577, 1498, 1456, 1328, 1168, 1058, 986, 846, 718, 672. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 5.51 (2H, s, CH_2), 6.88 (2H, d, ArH), 7.09 (1H, m, ArH), 7.27 (3H, m, ArH), 7.85 (2H, d, $J = 7.98$ Hz, ArH), 8.11 (2H, d, $J = 8.0$ Hz, ArH), 8.24 (1H, s, triazole-H), 9.42 (1H, s, triazole-H), *m/z*: 406.1 (M$^+$). Anal. calcd. for $C_{22}H_{14}F_3N_5$: C, 65.18; H, 3.48; N, 17.28; found C, 65.21; H, 3.51; N, 17.36.

*3.4.9. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-1-(3-chlorobenzyl)-4,6-difluoro-1H-benzo[d]imidazole (**10i**)*

The compound obtained as a pale yellow colour crystalline solid by using 1-(bromomethyl)-3-chlorobenzene (yield 71%). m.p. 127–129 °C. IR (KBr cm^{-1}): 3153, 2361, 1647, 1554, 1489, 1444, 1331, 1178, 1048, 991, 847, 722, 672. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 5.49 (2H, s, CH_2), 6.93 (2H, d, ArH), 7.12 (1H, m, ArH), 7.35 (3H, m, ArH), 7.77 (2H, d, $J = 7.96$ Hz, ArH), 8.10 (2H, d, $J = 7.98$ Hz, ArH), 8.26 (1H, s, triazole-H), 9.36 (1H, s, triazole-H), *m/z*: 422.1(M$^+$). Anal. calcd. for $C_{22}H_{14}ClF_2N_5$: C, 62.64; H, 3.35; N, 16.60; found C, 62.68; H, 3.51; N, 16.66.

*3.4.10. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-4,6-difluoro-1-(tosyl-methyl)-1H-benzo[d]imidazole (**10j**)*

The compound obtained as a pale yellow colour crystalline solid by using 4-methylbenzene-1-sulfonyl chloride (yield 73%); m.p. 182–184 °C. IR (KBr cm^{-1}): 3324, 3125, 2960, 1638, 1516, 1386, 1283, 1190, 1114, 948, 847, 669. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.64 (3H, s, CH_3), 7.12 (1H, m, ArH), 7.32 (1H, m, ArH), 7.48 (4H, d, ArH), 7.7 (2H, d, $J = 8.01$ Hz, ArH), 7.95 (2H, d, $J = 8.0$ Hz, ArH), 8.26 (1H, s, triazole-H), 9.36 (1H, s, triazole-H), *m/z*: 452.1 (M$^+$). Anal. calcd for $C_{22}H_{15}F_2N_5O_2S$: C, 58.53; H, 3.35; N, 15.51; found C, 58.34; H, 3.32; N, 15.50.

*3.4.11. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl) phenyl]-1-[3-(trifluoromethyl)-benzyl]-4,6-difluoro-1H-benzo[d]imidazole (**10k**)*

The compound obtained as a pale yellow colour crystalline solid by using 1-(bromomethyl)-3-(trifluoromethyl)-benzene (yield 77%). m.p. 133–135 °C. IR (KBr cm^{-1}): 3142, 2363, 1658, 1581, 1498, 1457, 1326, 1166, 1048, 985, 846, 718, 672. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 5.48 (2H, s, CH_2), 6.72 (2H, d, ArH), 6.88 (1H, m, ArH), 7.18 (3H, m, ArH), 7.68 (2H, d, $J = 8.02$ Hz, ArH), 7.98 (2H, d, $J = 8.05$ Hz, ArH), 8.14 (1H, s, triazole-H), 9.39 (1H, s, triazole-H), *m/z*: 456.1 (M$^+$). Anal. calcd. for $C_{23}H_{14}F_5N_5$: C, 60.66; H, 3.10; N, 15.38; found C, 60.72; H, 3.45; N, 15.39.

*3.4.12. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-1-(4-fluorobenzyl)-4,6-difluoro-1H-benzo[d]imidazole (**10l**)*

The compound obtained as a cremish colour crystalline solid by using 1-(bromomethyl)-4-fluorobenzene (yield 69%). m.p. 128–130 °C. IR (KBr cm^{-1}): 3138, 2361, 1665, 1574, 1497, 1460, 1326, 1154, 1055, 987, 844, 718, 672. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 5.51 (2H, s, CH_2), 6.90 (2H, d, ArH), 7.13 (2H, m, ArH), 7.31 (2H, m, ArH), 7.75 (2H, d, $J = 8.01$ Hz, ArH), 8.10 (2H, d, $J = 8.05$, Hz, ArH), 8.27 (1H, s, triazole-H), 9.48 (1H, s, triazole-H), *m/z*: 406.1 (M$^+$). Anal. calcd. for $C_{22}H_{14}F_3N_5$: C, 65.18; H, 3.48; N, 17.28; found C, 65.42; H, 3.64; N, 17.44.

*3.4.13. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-1-(3-methylbenzyl)-4,6-difluoro-1H-benzo[d]imidazole (**10m**)*

The compound obtained as a buff colour crystalline solid by using 1-(bromomethyl)-3-methylbenzene (yield 72%). m.p. 166–163 °C. IR (KBr cm^{-1}): 3098, 2363, 1663, 1574, 1511, 1464, 1338, 1188, 1044, 980, 847, 718, 671. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 2.78 (3H, s, CH_3) 5.49 (2H, s, CH_2), 6.84 (2H, d, ArH), 7.11 (1H, m, ArH), 7.27 (3H, m, ArH), 7.85 (2H, d, $J = 7.98$ Hz, ArH), 8.09 (2H, d, $J = 8.0$ Hz, ArH), 8.22 (1H, s, triazole-H), 9.48 (1H, s, triazole-H), *m/z*: 402.1 (M$^+$). Anal. calcd. for $C_{23}H_{17}F_2N_5$: C, 68.82; H, 4.27; N, 17.45; found C, 68.89; H, 4.51; N, 17.66.

*3.4.14. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl)phenyl]-1-(3-methoxybenzyl)-4,6-difluoro-1H-benzo[d]imidazole (**10n**)*

The compound obtained as a off-white colour crystalline solid by using 1-(bromomethyl)-3-methoxybenzene (yield 78%). m.p. 150–152 °C. IR (KBr cm^{-1}): 3141, 2362, 1648, 1581, 1511, 1461, 1332, 1172, 1066, 984, 847, 719, 673. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 3.98 (3H, s, OCH_3), 5.48 (2H, s, CH_2), 6.79 (2H, d, ArH), 7.10 (1H, m, ArH), 7.24 (3H, m, ArH), 7.79 (2H, d, $J = 7.92$ Hz, ArH), 8.10 (2H, d, $J = 7.96$ Hz, ArH), 8.22 (1H, s, triazole-H), 9.39 (1H, s, triazole-H), *m/z*: 418.1 (M$^+$). Anal. calcd for $C_{23}H_{17}F_2N_5O$: C, 66.18; H, 4.11; N, 16.78; found C, 66.23; H, 4.51; N, 16.90.

*3.4.15. Synthesis of 2-[4-(1H-[1,2,4]-triazol-1-yl) phenyl]-1-(2-methylbenzyl)-4,6-difluoro-1H-benzo[d]imidazole (**10o**)*

The compound obtained as a buff colour crystalline solid by using 1-(bromomethyl)-2-methylbenzene (yield 79%). m.p. 134–136 °C. IR (KBr cm^{-1}): 3121, 2359, 1656, 1578, 1510, 1438, 1351, 1179, 1055, 981, 846, 717, 672. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 2.68 (3H, s, CH_3) 5.51 (2H, s, CH_2), 6.87 (2H, d, ArH), 7.11 (2H, m, ArH), 7.24 (2H, m, ArH), 7.78 (2H, d, $J = 7.92$ Hz, ArH), 8.01 (2H, d, $J = 7.93$ Hz, ArH), 8.22 (1H, s, triazole-H), 9.42 (1H, s, triazole-H). *m/z*: 402.1 (M$^+$). Anal. calcd. for $C_{23}H_{17}F_2N_5$: C, 68.82; H, 4.27; N, 17.45; found C, 68.89; H, 4.51; N, 17.66.

3.5. Pharmacological activity

3.5.1. Antitubercular activity

Primary screening was conducted at 6.25 µg/mL^{-1} against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the microplate Alamar Blue assay (MABA) [36]. Compounds exhibited fluorescence were tested in the BACTEC 460 radiometric system [37]. Compounds showing more than 95% inhibition in the primary screening were considered active and again retested at lower concentration 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 (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.

3.5.2. *Antimicrobial activity*

The compounds listed in were screened for the antimicrobial activity against different gram +ve organisms *S. aureus*, *P. aeruginosa* and gram −ve organisms *E. coli* and *S. typhosa* under the following conditions (Table 3).

The test was performed according to the agar well diffusion method [38] adopted with some modifications for the synthesized compounds using Gentamycin as a reference standard. In this method, the wells were punched in the agar plates using a sterile stainless steel borer (10 mm diameter). The bacterial strains were maintained on the agar medium and the impregnated disk incubated in the incubator at 24–28 °C for 24 h. After 24 h, the sterile disks were impregnated with different compounds dissolved in dimethyl sulfoxide having 100-μl concentration of each compound with varying concentrations ranging from 0.5% to 1.0%. The impregnated disk incubated at 24–28 °C for 24 h for bacterial inhibition. The inhibition zones caused by the various compounds on the microorganisms were examined. The diameters of the inhibitory zone were measured in mm.

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