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3D-QSAR DRIVEN DESIGN, SYNTHESIS AND EVALUATION OF NOVEL BIPHENYL DERIVATIVES OF BENZIMIDAZOLE (PA-824 ANALOGUES) AS ANTI-TUBERCULAR AGENTS

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ABSTRACT

A series of novel biphenyl derivatives of benzimidazole (PA-824 analogues) as anti-tubercular agents was synthesised, characterised and evaluated for their anti-mycobacterial activity, by using an *in silico* design and 3D-QSAR-driven approach. Initially, we developed SAR rules and 3D-QSAR models using literature data for targeted design of new biphenyl derivatives of PA-824 analogues with anti-TB activity. Using these models, we prioritized 12 compounds for synthesis and biological evaluation. As a result compound 6c & 6d were 2.5 and 2 fold more active against *M. tuberculosis* when compared to the standard drug RMP (MICs of 0.04 and 0.05 µg mL-1 respectively) while compound 6e & 6f having 3-fluoro-4-methoxy and 3-fluoro-4-trifluoromethoxy groups at ring B were found to be equally potent to standard drug RMP (MICs of 0.09 and 0.12 µg mL-1 respectively).

KEYWORDS: 3D-QSAR**,** *Mycobacterium tuberculosis*, tuberculosis, drug design, benzimidazole, PA-824.

INTRODUCTION

The causative agent for the disease Tuberculosis (TB) is *Mycobacterium tuberculosis.* Primarily lungs are the affected by the disease but any organ of the body can be attacked and spread from one person to another through air. Primary symptoms of infection are coughs or sneezing. However, everyone infected with TB bacteria does not become sick. Two main TB-related conditions are there: latent TB infection and active TB disease. Both of these conditions are treatable and curable.[1] Despite of the thousands of the years of recognition of

the disease and the causative agent Tuberculosis has been one of the largest infectious diseases since the earliest days of medical microbiology.[2] World Health Organisation (WHO) reported about 9.2 million people infected by TB and 1.7 million deaths due to TB every year. More over due to the Human immunodeficiency virus emergence it has become the most susceptible infection to the patients suffering from AIDS. There are around 0.7 million HIV-positive people infected with TB, contributing to 0.2 million deaths worldwide per year. The lethal combination of TB and HIV is fuelling the TB epidemic in many parts of the world.[3] Population growth, poor case detection and low cure rates in non-developed countries, transmission in overcrowded hospitals, prisons and other public places, drug abuse and homelessness are other factors which contributed.[4]

Standard short course regimens and directly observed therapy (DOTS) have improved response rates, MDR isolates and extensively resistant (XRD) TB isolates continues to emerge. The unusual microbiological characteristics of *Mycobacterium tuberculosis* organism such as long generation time, ability to enter a dormant state, persistence in the face of appropriate therapy, strong protective cell wall and the ability to undergo genetic mutation to develop resistance to drugs are the present challenges for development of new drugs.[2] Chemotherapy has been evolved the most common potent and useful tool for treatment and prognosis of tuberculosis and has almost reached the goal of eradicating tuberculosis from some western countries.[5]

The benzimidazole derivatives are used as antimicrobial agents against the wide spectrum of microorganism. Due to its synthetic utility and broad range of pharmacological effects, the benzimidazole nucleus is an important heterocyclic ring, in organic chemistry, synthesis and microbiology of this pharmacophore continues to be fuelled by its antifungal, antitubercular, antioxidant and anti-allergic properties. Other reports have revealed that these molecules are also present in a variety of antiparasitic and herbicidal agents.[6-7] Albendazole, fenbendazole, sulphoxide derivatives and methylcarbamate benzimidazoles with a broad spectrum anthelmentic activity, are most common example of drugs widely used in human and veterinary medicine and also used against several systemic parasitoses, including nematodoses, hidatidosis, teniasis and others. They are also used to treat microsporodial and cryptosporodial infections which can cause lethal diarrhea in patients treated with immunosuppressive drugs, or infected with HIV.[7]

Many of Heterocyclic compounds bearing nitrogen, sulphur and oxygen constitute the core structure of the compounds for chemotherapeutic use.[8] During last decades lot benzimidazoles nucleus derivatives have been synthesised and studied extensively. A large number of biological activities of these derivatives have been reported in the literature possess that include anti-cancer,[9-11] antimalarial,[12] antiviral,[13-14] antimicrobial,[15-17] analgesic & anti-inflammatory,[18-19] antihypertensive,[20] anticonvulsant,[21] antifungal,[22] antiasthamatic,[23] antidiabetic,[23] antioxidant[24] and antiprotozoal[25] activities. Owing to the importance of benzimidazole, now we wish to describe the SAR studies of benzimidazole and their in vitro screening results of antitubercular activities. Some nitroimidazole based antimycobacterial drugs are in clinical evaluation (Fig. 1).

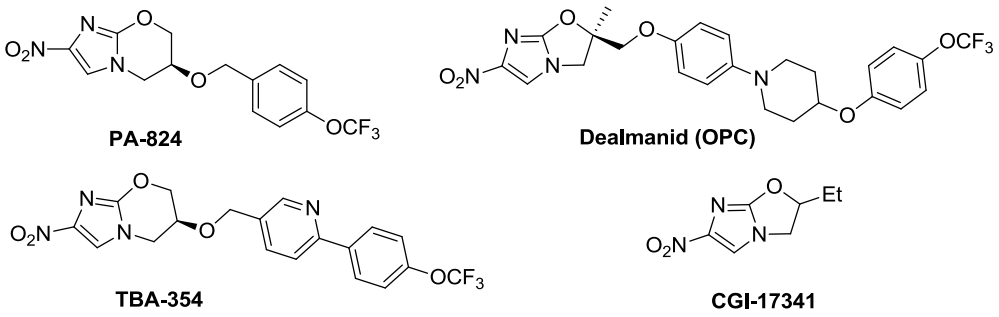


Fig. 1: Structures of nitroimidazole based antitubercular agents.

The goal of this work was to design, synthesis and evaluation of novel biphenyl derivatives of benzimidazole with potent anti-TB activity. To achieve this goal, we performed the following steps: (i) collection of available data and rigorous data curation; (ii) generation of common pharmacophore hypotheses; (iii) development 3D QSAR model and of design novel biphenyl derivatives of benzimidazole with potential anti-TB activity; (iv), organic synthesis and structure identification (NMR, MS, and IR) of selected 3D QSAR hits; and (vi) *in vitro* experimental evaluation of designed compounds (MABA).

RESULT AND DISCUSSION

Pharmacophore model generation & ligand based 3D-QSAR model generation

A 3D-QSAR study was successfully performed on the series of biphenyl derivatives of benzimidazole to understand the effect of spatial arrangement of structural features on anti-mycobacterium activities (MABA). Results of the 3D-QSAR are presented in our previous publication. [26] The 2D representation of the hypothesis is given in Fig 2.

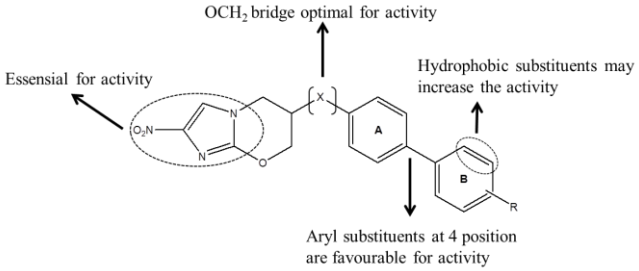
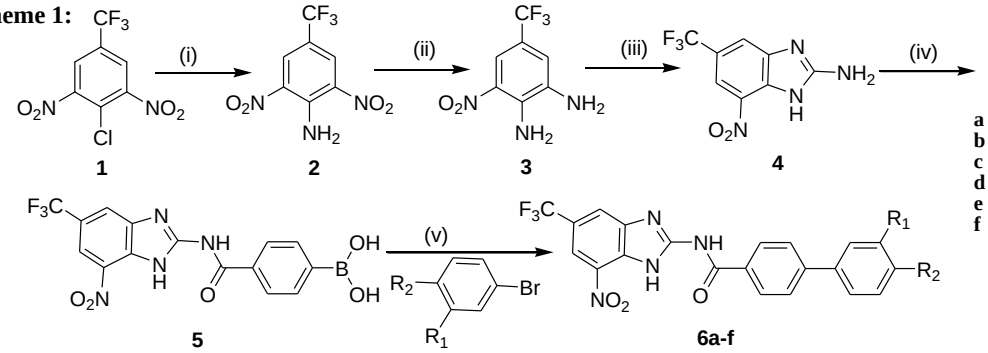


Fig. 2: Design strategy for novel PA-824 derivatives.

Chemistry

Based on the results of the 3D QSAR, we synthesized the selected novel biphenyl derivatives of benzimidazole. Compounds 6a-f were synthesised as per the procedure described in the chemistry section (Scheme 1) while compounds 12a, 12c and 13c-f were synthesised as per the procedure described in scheme 2. Spectral data of synthesised compounds corroborated well with the proposed structures and are given in the experimental section.

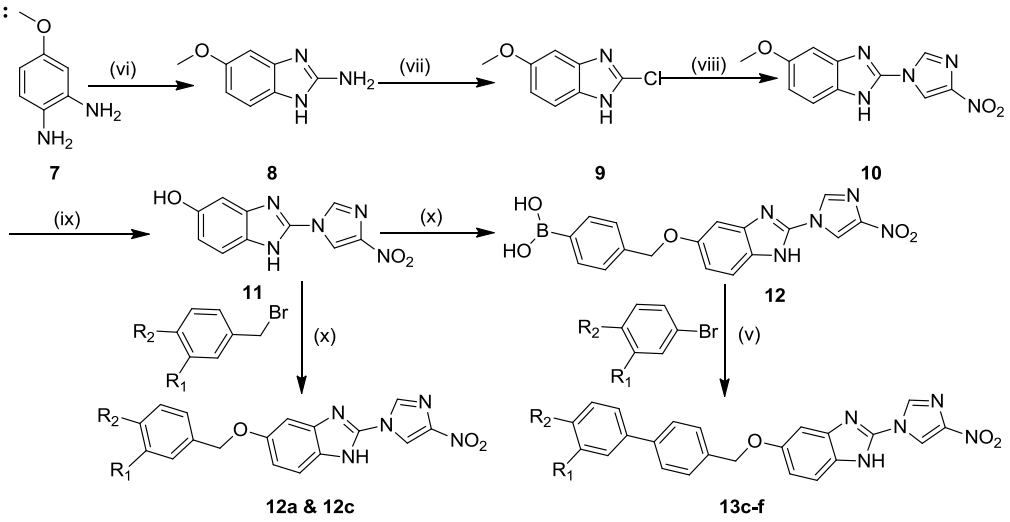
Scheme 1:



	R1	R2
a	H	CF3
b	H	OMe
c	H	CN
d	F	CF3
e	F	OMe
f	F	OCF3

Reagents and conditions : (i) NH_3/EtOH, 120^o C, 6h (ii) 10% Pd/C, EtOH, 6h (iii) CNBr, MeOH/H_2O, 60^o C, 5h (iv) EDCI, HOBT, Et_3N, DMF, RT, 6h (v) $PdCl_2$(dppf), K_2CO_3, EtOH/Toluene, reflux, 5h

Scheme 2 :



Reagents and conditions : (vi) CNBr, $NaHCO_3$, ACN, RT, 48 h (vii) $CuCl_2$, t-BuONO, ACN, 60^o C, 6h (viii) Cs_2CO_3, DMF, 90^o C, 16 h (ix) BBr_3, DCM, RT, 12 h (x) K_2CO_3, acetone, 40^o C, 12 h

Biological evaluation

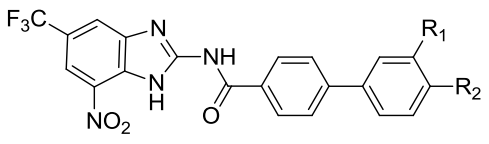
Antituberculosis activity

The compounds were submitted for assessment of their antitubercular activity using Middle brook 7H9 broth against *Mycobacterium tuberculosis* of H37Rv strain under *aerobic* (replicating) condition using MABA assays.[27] Minimum inhibitory concentrations (MICs) were defined as the lowest compound concentration effecting ≥90% inhibition of fluorescence. We evaluated 12 compounds with known standard drug Rifampicin (RMP). MIC's of the compounds were reported in Table 1.

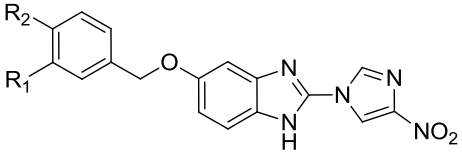
Among the synthesized compounds, four compounds were found to be most active with minimum inhibitory concentration less than 0.1µg mL^{-1}. Compounds with 4-cyano and 3-fluoro-4-trifluoromethyl group substitutions at ring B (6c and 6d) were 2.5 and 2 fold more active against *M. tuberculosis* when compared to the standard drug RMP (MICs of 0.04 and 0.05 µg mL^{-1} respectively) while compound 6e and 6f having 3-fluoro-4-methoxy and 3-fluoro-4-trifluoromethoxy groups at ring B were found to be equally potent to standard drug RMP (MICs of 0.09 and 0.12 µg mL^{-1} respectively).

Four compounds (6c, 6d, 6e and 6f) were further examined for toxicity (IC50) in a mammalian VERO cell line at concentrations of 62.5 µg mL^{-1}. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 nonradioactive cell proliferation assay.[28] The compounds were found to be nontoxic till 62.5 µg mL^{-1}.

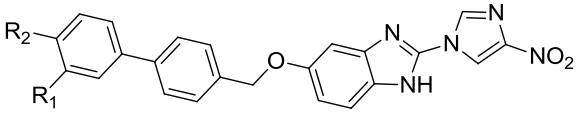
Table 1: Antimycobacterial activity of the synthesized compounds.



6a-f



12a & 12c



13c-f

S. No.	Compound	X	R1	R2	M. Wt.	MIC (µg mL-1)
1.	6a	NHCO	H	CF_3	494.35	3.25
2.	6b	NHCO	H	OMe	456.37	1.42
3.	6c	NHCO	H	CN	451.36	0.04
4.	6d	NHCO	F	CF_3	512.34	0.05
5.	6e	NHCO	F	OMe	474.36	0.12
6.	6f	NHCO	F	OCF_3	528.34	0.09
7.	13c	OCH_2	H	CN	436.4	4.67
8.	13d	OCH_2	F	CF_3	497.4	3.52
9.	13e	OCH_2	F	OMe	459.4	1.64
10.	13f	OCH_2	F	OCF_3	513.3	0.95
11.	12a	OCH_2	H	CF_3	403.3	1.76
12.	12c	OCH_2	H	CN	360.3	2.53
13.	RMP	-	-	-	822.9	0.1

MATERIALS AND METHODS

Experimental

Chemicals and solvents were procured from CDH, E. Merck, SD Fine, and Combi blocks, GLR, Spectrochem, Finar and used as such without further purification. Melting points were determined by open capillary tube method and are uncorrected. Homogeneity of the compounds was checked on Merck precoated silica gel plates using iodine vapours, ninhydrin stain, KMnO4 stain and 2,4-DNP as visualising agents. Compounds were characterised by their mass and 1H NMR (Bruker 400 MHz). Mass spectra were recorded on Shimadzu, mass spectrometer.

Synthesis of 2,6-dinitro-4-(trifluoromethyl)benzenamine (2)

2-chloro-1,3-dinitro-5-(trifluoromethyl)benzene (5 mmol) and ammonical ethanol (20 mL) were charged to a closed vessel and heated to 120^o C for 6 h. Progress of reaction was monitored by TLC. After reaction completion solvent was removed under reduced pressure. Crude was purified by column chromatography using silica gel (100-200#) as stationary phase and 40 % EtOAc/hexane as eluent to give the title compound as yellow solid. Yield-76%; MP 143-144^o C; ^{1}H NMR (400 MHz, CDCl3) δ 7.3 (s, 2H), 4.56 (brs, 2H), MS (ESI) *m/z:* 252 $[M + H]^+$ Elem. Anal. calculated for $C_7H_4F_3N_3O_4$: C, 33.48; H, 1.61; F, 22.70; N, 16.73; O, 25.48 Found : C, 33.53; H, 1.59; F, 22.89; N, 16.69; O, 25.53.

Synthesis of 3-nitro-5-(trifluoromethyl)benzene-1,2-diamine (3)

To a solution of 2,6-dinitro-4-(trifluoromethyl)benzenamine (5 mmol) in EtOH (30 mL) was added 10% Pd/C (0.2 g) and hydrogenated for 6 h. Progress of reaction was monitored by TLC. After reaction completion mixture was filtered through celite pad and the filtrate was

evaporated under reduced pressure. Crude was purified by column chromatography using silica gel (100-200#) as stationary phase and 30 % Acetone/hexane as eluent to give the title compound as yellow solid. Yield-67%; MP 121-123^{o} C; ^{1}H NMR (400 MHz, DMSO) δ 7.86 (d, 1H, J=1.9 Hz), 6.78 (d, 1H, J=1.9 Hz), 4.58 (brs, 2H), 3.84 (brs, 2H), MS (ESI) *m/z:* 222 $[M + H]^{+}$ Elem. Anal. calculated for $C_7H_6F_3N_3O_2$: C, 38.02; H, 2.73; F, 25.77; N, 19.00; O, 14.47Found : C, 38.13; H, 2.63; F, 25.67; N, 19.00; O, 14.42.

Synthesis of 7-nitro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-amine (4)

To a solution of compound 3-nitro-5-(trifluoromethyl)benzene-1,2-diamine (1 mmol) in 1:1 mixture of MeOH (10 mL) and water (10 mL) was added CNBr (3 mmol). The reaction mixture was stirred for 5 h at 60 ºC. The reaction mixture was cooled to room temperature; MeOH was removed under reduced pressure, and the remaining mixture was basified with 1.0 M aq. NaOH (to pH = 8.0) and extracted with EtOAc (3 × 30 mL). The combined organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude material was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to obtain the title compound as a white solid. Yield-82%; MP 267-269^{o} C; ^{1}H NMR (400 MHz, DMSO) δ 9.6 (s, 2H), 8.1 (brs, 1H), 8.05 (d, 1H, J=1.9 Hz), 7.63 (d, 1H, J=1.9 Hz). MS (ESI) *m/z:* 247.08 $[M + H]^{+}$ Elem. Anal. calculated for $C_8H_5F_3N_4O_2$: C, 39.04; H, 2.05; F, 23.16; N, 22.76; O, 13.00 Found : C, 38.97; H, 2.01; F, 23.11; N, 22.72; O, 13.02.

Synthesis of 4-((7-nitro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)carbamoyl)phenylboronic acid (5)

To a solution of 4-boronobenzoic acid (3mmol) in DMF (10 mL) were added 7-nitro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-amine (3 mmol), EDCI (4.5 mmol), HOBT (4.5 mmol) and trimethylamine (9 mmol). The resulting solution was stirred at RT for 6h. Progress of reaction was monitored by TLC. After reaction completion RM was cooled to RT, added water and extracted with ethyl acetate (3 X 10 mL). Combined organic washed with brine, dried over sodium sulphate and concentrated under reduced pressure. The crude material was stirred with 20 % diethyl ether in hexane for 3 h, filtered and dried to obtain the title compound as a yellow solid. Yield-68%; MP 287-280^{o} C; ^{1}H NMR (400 MHz, DMSO) δ 10.6 (brs, 1H), 8.61 (d, 1H, J = 1.9 Hz), 8.32 (s, 2H), 8.18 (brs, 1H), 8.11 (d, 1H, J = 1.9 Hz), 7.81 (dd, 2H, J = 7.8, 1.8 Hz), 7.65 (dd, 2H, J = 7.8, 1.4 Hz), MS (ESI) m/z: 395.15 [M + H]+ Elem. Anal. calculated for C15H10BF3N4O5 : C, 39.04; H, 2.05; F, 23.16; N, 22.76; O, 13.00 Found : C, 38.97; H, 2.01; F, 23.11; N, 22.72; O, 13.02 .

General procedure for Suzuki reaction:

A mixture of 4-((2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazol-5-yloxy)methyl)phenylboronic acid (5 mmol) and aryl bromide (6 mmol) in aqueous K_2CO_3 (2M, 15 mmol), EtOH(15 mL), and toluene (25 mL) was purged with N_2. $Pd(dppf)Cl_2$ (0.25 mmol) was added, and the mixture was refluxed under N_2 for 5 h and then partitioned between EtOAc and 0.1MHCl. Crude was purified by column chromatography using silica gel (100-200#) as stationary phase and 40 % Acetone/hexane as eluent to give the desired product. The same experimental procedure was followed for the preparation of compounds 6a-f and 13c-f.

4-(4'-(trifluoromethyl)phenyl)-N-(7-nitro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)benzamide (6a)

Yield-61%; MP 188-190^o C; ^{1}H NMR (400 MHz, DMSO) δ 10.1 (brs, 1H), 8.61 (d, 1H, *J* = 1.9 Hz), 8.21 (brs, 1H), 8.11 (d, 1H, *J* = 1.9 Hz), 7.86 (dd, 2H, *J* = 8.7, 1.9 Hz), 7.76-7.86 (m, 4H), 7.83 (dd, *J* = 8.7, 1.5 Hz), 7.70 (dd, 2H, *J* = 8.7, 1.7 Hz). MS (ESI) *m/z:* 495.25 $[M + H]^+$ Elem. Anal. calculated for $C_{22}H_{12}F_6N_4O_3$: C, 53.45; H, 2.45; F, 23.06; N, 11.33; O, 9.71Found : C, 53.41; H, 2.42; F, 23.01; N, 11.23; O, 9.61.

4-(4'-methoxyphenyl)-N-(7-nitro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)benzamide (6b)

Yield-54%; MP 164-166^o C; ^{1}H NMR (400 MHz, DMSO) δ 10.2 (brs, 1H), 8.61 (d, 1H, *J* = 1.9 Hz), 8.16 (brs, 1H), 8.11 (d, 1H, *J* = 1.9 Hz), 7.60-7.71 (m, 4H), 7.53 (dd, 2H, *J* = 9.0, 1.5 Hz), 6.97 (dd, 2H, *J* = 9.0, 1.6 Hz), 3.81 (s, 3H). MS (ESI) *m/z:* 457.17 $[M + H]^+$ Elem. Anal. calculated for $C_{22}H_{15}F_3N_4O_4$: C, 57.90; H, 3.31; F, 12.49; N, 12.28; O, 14.02 Found : C, 57.86; H, 3.21; F, 12.41; N, 12.22; O, 14.06.

4-(4'-cyanophenyl)-N-(7-nitro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)benzamide (6c)

Yield-61%; MP 163-166^o C; ^{1}H NMR δ 10.1 (brs, 1H), 8.61 (d, 1H, *J* = 1.9 Hz), 8.19 (brs, 1H), 8.11 (d, 1H, *J* = 1.9 Hz), 8.01 (dd, 2H, *J* = 8.7, 1.5 Hz), 7.81-7.92 (m, 4H), 7.83 (dd, 2H, *J* = 8.7, 1.5 Hz). MS (ESI) *m/z:* 452.26 $[M + H]^+$ Elem. Anal. calculated for $C_{22}H_{12}F_3N_5O_3$: C, 58.54; H, 2.68; F, 12.63; N, 15.52; O, 10.63 Found : C, 58.44; H, 2.61; F, 12.54; N, 15.46 O, 10.53.

4-(3'-fluoro-4'-(trifluoromethyl)phenyl)-N-(7-nitro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)benzamide (6d)

Yield-56%; MP 182-184^{o} C; ^{1}H NMR (400 MHz, DMSO) δ 10.2 (brs, 1H), 8.61 (d, 1H, *J* = 1.9 Hz), 8.18 (brs, 1H), 8.11 (d, 1H, *J* = 1.9 Hz), 8.06 (dd, 1H, *J* = 1.6 Hz), 7.76-7.87 (m, 3H), 7.71 (dd, 2H, *J* = 8.7, 1.7 Hz), 7.51 (dd, 1H, *J* = 8.7, 1.6 Hz). MS (ESI) *m/z:* 513.14 [M + H]$^{+}$ Elem. Anal. calculated for $C_{22}H_{11}F_7N_4O_3$: C, 51.57; H, 2.16; F, 25.96; N, 10.94; O, 9.37 Found : C, 51.51; H, 2.11; F, 25.86; N, 10.86; O, 9.27.

4-(3'-fluoro-4'-methoxyphenyl)-N-(7-nitro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)benzamide (6e)

Yield-63%; MP 181-183^{o} C; ^{1}H NMR (400 MHz, DMSO) δ 10.2 (brs, 1H), 8.61 (d, 1H, *J* = 1.9 Hz), 8.11 (d, 1H, *J* = 1.9 Hz), 8.20 (brs, 1H), 7.75 (dd, 1H, *J* = 1.5, 0.5 Hz), 7.60-7.73 (m, 4H), 7.36 (dd, 1H, *J* = 8.9, 1.5 Hz), 6.92 (dd, 1H, *J* = 8.9, 0.5 Hz), 3.79 (s, 3H). MS (ESI) *m/z:* 475.16 [M + H]$^{+}$ Elem. Anal. calculated for $C_{22}H_{14}F_4N_4O_4$: C, 55.70; H, 2.97; F, 16.02; N, 11.81; O, 13.49Found : C, 55.62; H, 2.87; F, 15.92; N, 11.71; O, 13.42.

4-(3'-fluoro-4'-(trifluoromethoxy)phenyl)-N-(7-nitro-5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl)benzamide (6f)

Yield-51%; MP 168-170^{o} C; ^{1}H NMR (400 MHz, DMSO) δ 10.2 (brs, 1H),8.61 (d, 1H, *J* = 1.9 Hz), 8.25 (brs, 1H), 8.11 (d, 1H, *J* = 1.9 Hz), 7.76-7.82 (m, 3H), 7.67 (dd, 2H, *J* = 8.7, 1.6 Hz), 7.51 (dd, 1H, *J* = 8.9, 0.5 Hz), 7.25 (dd, 1H, *J* = 8.9, 1.5 Hz). MS (ESI) *m/z:* 529.04 [M + H]$^{+}$ Elem. Anal. calculated for $C_{22}H_{11}F_7N_4O_4$: C, 50.01; H, 2.10; F, 25.17; N, 10.60; O, 12.11Found : C, 49.06; H, 2.02; F, 25.07; N, 10.55; O, 12.01.

Synthesis of 5-Methoxy-2-aminobenzimidazole (8)

A solution of 4-methoxy-1,2-phenylenediamine dihydrochloride (2.5 mmol) in 5 mL of water was cooled to 0 °C and treated with a solution of cyanogen bromide (0.60 mL, 5 M in acetonitrile, 3.2 mmol) and solid $NaHCO_3$ (5 mmol). The deep purple solution was stirred at ambient temperature for 48 h. The mixture was made basic with 1 M aqueous Na_2CO_3; then the solution was concentrated under reduced pressure. The residue was triturated with hot ethanol (20 mL), and the ethanol solution was filtered and concentrated under reduced pressure to give the title compound as a tan solid (90%). MP 198-200^{o}C; ^{1}H NMR (400 MHz, DMSO) δ 8.0-9.0 (brs, 1H), 7.52 (brs, 2H), 7.16 (d, 1H, *J* = 8.0), 6.85 (d, 1H, *J* = 2), 6.7 (dd, 1H, *J* = 8.5, 1.5 Hz), 3.65 (s, 3H). MS (ESI) *m/z:* 164 [M + H]$^{+}$ Anal. calculated for $C_8H_9N_3O$: C, 58.88; H, 5.56; N, 25.75; O, 9.80 Found : C, 58.78; H, 5.56; N, 25.65; O, 9.72.

Synthesis of 2-chloro-5-methoxy-1H-benzo[d]imidazole (9)

To a suspension of $CuCl_2$ (3.4 mmol) in ACN (8 mL) was added t-BuONO (3.9 mmol). The mixture was stirred for 5 min then a solution of 5-Methoxy-2-aminobenzimidazole (2.8 mmol) in ACN (8 mL) was added dropwise. The reaction mixture was stirred at 60^o C for 3h. Progress of reaction was monitored by TLC. After reaction completion RM was cooled to RT, added water and extracted with ethyl acetate (3 X 10 mL). Combined organic washed with brine, dried over sodium sulphate and concentrated under reduced pressure and the crude material was purified by silica gel column chromatography (20 % Acetone/hexane) to obtain the title compound as a yellow oil (67 %). BP 365^{o}C; ^{1}H NMR (400 MHz, DMSO) δ 11.8 (brs, 1H), 7.14 (dd, 1H, *J* = 8.5, 0.4 Hz), 6.88 (dd, 1H, *J* = 1.4, 0.4 Hz), 6.66 (dd, 1H, *J* = 8.5, 1.4 Hz), 3.58 (3H, s). MS (ESI) *m/z:* 183.5 $[M + H]^+$ Anal. calculated for $C_8H_7ClN_2O$: C, 52.62; H, 3.86; Cl, 19.41; N, 15.34; O, 8.76 Found : C, 52.57; H, 3.78; Cl, 19.36; N, 15.29; O, 8.66.

Synthesis of 5-methoxy-2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazole (10)

To a solution of 4-nitro-1H-imidazole (3.3 mmol) in DMF (5 mL) was added Cs_2CO_3 (9 mmol) and stirred for 15 min. Solution of 2-chloro-5-methoxy-1H-benzo[d]imidazole (3 mmol) in DMF (2 mL) was then added and the resulting mixture was then heated to 90^o C for 16h. Progress of reaction was monitored by TLC. After reaction completion RM was cooled to RT, added water and extracted with ethyl acetate (3 X 10 mL). Combined organic washed with brine, dried over sodium sulphate and concentrated under reduced pressure to obtain the title compound as a yellow solid (84 %). MP 276-278^{o}C; ^{1}H NMR (400 MHz, DMSO) δ 10.9 (brs, 1H), 8.63 (d, 1H, *J* = 1.8 Hz), 8.33 (d, 1H, *J* = 1.8 Hz), 7.57 (dd, 1H, *J* = 8.2, 0.4 Hz), 7.21 (dd, 1H, *J* = 1.6, 0.4 Hz), 6.74 (dd, 1H, *J* = 8.2, 1.6 Hz), 3.68 (s, 3H). MS (ESI) *m/z:* 260.2 $[M + H]^+$ Anal. calculated for $C_{11}H_9N_5O_3$: C, 50.97; H, 3.50; N, 27.02; O, 18.52 Found : C, 50.87; H, 3.45; N, 26.93; O, 18.42.

Synthesis of 2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazol-5-ol (11)

To a solution of 5-methoxy-2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazole (2.5 mmol) in DCM was added BBr_3 (1M in DCM, 4.8 mmol) at 0^{o}C over 10 min. and stirred overnight at RT. After reaction completion RM was concentrated and the residue stirred with ice/water. Solids were filtered, washed with water, hexane and dried to provide the product as yellow solid (72 %). MP 166-168^{o}C; ^{1}H NMR (400 MHz, DMSO) δ 10.9 (brs, 1H), 8.63 (d, 1H, *J* = 1.8 Hz), 8.33 (d, 1H, *J* = 1.8 Hz), 7.36 (dd, 1H, *J* = 8.2, 0.4 Hz), 7.17 (dd, 1H, *J* = 1.6, 0.4

Hz), 6.92 (dd, 1H, *J* = 8.2, 1.6 Hz), 5.3 (brs, 1H). MS (ESI) *m/z:* 246.2 $[M + H]^+$ Anal. calculated for $C_{10}H_7N_5O_3$: C, 48.98; H, 2.88; N, 28.56; O, 19.58 Found : C, 48.88; H, 2.81; N, 28.47; O, 19.46.

General procedure for O-alkylation

To a solution of 2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazol-5-ol (5 mmol) in acetone (30 mL) was added K_2CO_3 (15 mmol) and stirred for 15 min. Substituted benzyl halide (6 mmol) was then added and the mixture attired overnight at 40^o C. Progress of reaction was monitored by TLC. After reaction completion RM was cooled to RT, added water and extracted with ethyl acetate (3 X 10 mL). Combined organic washed with brine, dried over sodium sulphate and concentrated under reduced pressure and the crude material was purified by stirring with 20 % diethyl ether in hexane for 3 h, filtered and dried to obtain the title compound as a yellow solid.

4-((2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazol-5-yloxy)methyl)phenylboronic acid (12)

Yield-68%; MP 248-250^o C; ^{1}H NMR (400 MHz, DMSO) δ 10.8 (brs, 1H), 8.63 (d, 1H, *J* = 1.8 Hz), 8.33 (d, 1H, *J* = 1.8 Hz), 8.12 (s, 2H),7.64 (dd, 1H, *J* = 8.2, 0.5 Hz), 7.54 (dd, 2H, *J* = 8.3, 1.2 Hz), 7.38 (dd, 2H, *J* = 8.3, 1.2 Hz), 7.24 (dd, 1H, *J* = 1.6, 0.5 Hz), 6.80 (dd, 1H, *J* = 8.2, 1.6 Hz), 5.29 (s, 2H,). MS (ESI) *m/z:* 380.16 $[M + H]^+$ Elem. Anal. calculated for $C_{17}H_{14}BN_5O_5$: C, 53.85; H, 3.72; B, 2.85; N, 18.47; O, 21.10 Found : C, 53.75; H, 3.67; B, 2.76; N, 18.4; O, 21.02.

5-(4-(trifluoromethyl)benzyloxy)-2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazole (12a)

Yield-71%; MP 190-192^o C; ^{1}H NMR (400 MHz, DMSO) δ 10.8 (brs, 1H), 8.63 (1H, d, *J* = 1.8 Hz), 8.33 (1H, d, *J* = 1.8 Hz), 7.57-7.69 (m, 5H), 7.24 (1H, dd, *J* = 1.6, 0.5 Hz), 6.80 (1H, dd, *J* = 8.2, 1.6 Hz), 5.28 (2H, s). MS (ESI) *m/z:* 404.3 $[M + H]^+$ Elem. Anal. calculated for $C_{18}H_{12}F_3N_5O_3$: C, 53.60; H, 3.00; F, 14.13; N, 17.36; O, 11.90 Found : C, 53.54; H, 2.92; F, 14.07; N, 17.30; O, 11.80.

4-((2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazol-5-yloxy)methyl)benzonitrile (12c)

Yield-74%; MP 182-184^o C; ^{1}H NMR (400 MHz, DMSO) δ 10.8 (brs, 1H), 8.63 (d, 1H, *J* = 1.8 Hz), 8.33 (d, 1H, *J* = 1.8 Hz), 7.85 (dd, 2H, *J* = 8.2, 1.9 Hz), 7.64 (dd, 1H, *J* = 8.2, 0.5 Hz), 7.33 (dd, 2H, *J* = 8.2, 1.5 Hz), 7.24 (dd, 1H, *J* = 1.6, 0.5 Hz), 6.80 (dd, 1H, *J* = 8.2, 1.6

Hz), 5.26 (s, 2H). MS (ESI) *m/z:* 361.3 $[M + H]^+$ Elem. Anal. calculated for $C_{18}H_{12}N_6O_3$: C, 60.00; H, 3.36; N, 23.32; O, 13.32 Found : C, 659.04; H, 3.31; N, 23.27; O, 13.26.

4-(4-((2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazol-5-yloxy)methyl)phenyl) benzonitrile (13c)

Yield-64%; MP 198-200^o C; ^{1}H NMR (400 MHz, DMSO) δ 10.8 (brs, 1H), 8.63 (d, 1H, *J* = 1.8 Hz), 8.33 (d, 1H, *J* = 1.8 Hz), 7.84 (ddd, 2H, *J* = 8.6, 1.3, 0.5 Hz), 7.72-7.79 (m, 4H), 7.61-7.68(m, 3H), 7.24 (dd, 1H, *J* = 1.6, 0.5 Hz), 6.81 (dd, 1H, *J* = 8.2, 1.6 Hz), 5.22 (s ,2H). MS (ESI) *m/z:* 437.4 $[M + H]^+$ Elem. Anal. calculated for $C_{24}H_{16}N_6O_3$: C, 66.05; H, 3.70; N, 19.26; O, 11.00 Found : C, 65.93 H, 3.65; N, 19.20; O, 10.94.

5-(4-(3-fluoro-4-(trifluoromethyl)phenyl)benzyloxy)-2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazole (13d)

Yield-61%; MP 202-204^o C; ^{1}H NMR (400 MHz, DMSO) δ 10.8 (brs, 1H), 8.63 (d, 1H, *J* = 1.8 Hz), 8.33 (d, 1H, *J* = 1.8 Hz), 8.02 (dd, 1H, *J* = 1.9, 0.5 Hz), 7.82 (ddd, 2H, *J* = 8.5, 1.1, 0.5 Hz), 7.73 (dd, 1H, *J* = 8.7, 0.5 Hz), 7.61-7.67 (m, 3H), 7.49 (dd, 1H, *J* = 8.7, 1.9 Hz), 7.24 (dd, 1H, *J* = 1.6, 0.5 Hz), 6.81 (dd, 1H, *J* = 8.2, 1.6 Hz), 5.23 (2H, s). MS (ESI) *m/z:* 498.4 $[M + H]^+$ Elem. Anal. calculated for $C_{24}H_{15}F_4N_5O_3$: C, 57.95; H, 3.04; F, 15.28; N, 14.08; O, 9.65 Found : C, 57.90; H, 3.14; F, 15.21; N, 14.02; O, 9.60.

5-(4-(3-fluoro-4-methoxyphenyl)benzyloxy)-2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazole (13e)

Yield-66%; MP 178-181^o C; ^{1}H NMR (400 MHz, DMSO) δ 10.8 (brs, 1H), 8.63 (d, 1H, *J* = 1.8 Hz), 8.33 (d, 1H, *J* = 1.8 Hz), 7.62-7.74 (m, 4H), 7.36-7.49 (m, 3H), 7.24 (dd, 1H, *J* = 1.6, 0.5 Hz), 6.93 (dd, 1H, *J* = 8.8, 0.5 Hz), 6.81 (dd, 1H, *J* = 8.2, 1.6 Hz), 3.88 (s,3H), 5.07 (s, 2H).MS (ESI) *m/z:* 460.4 $[M + H]^+$ Elem. Anal. calculated for $C_{24}H_{18}FN_5O_4$: C, 62.74; H, 3.95; F, 4.14; N, 15.24; O, 13.93 Found : C, 62.68; H, 3.90; F, 4.07; N, 15.15; O, 13.85.

5-(4-(3-fluoro-4-(trifluoromethoxy)phenyl)benzyloxy)-2-(4-nitro-1H-imidazol-1-yl)-1H-benzo[d]imidazole (13f)

Yield-63%; MP 185-187^o C; ^{1}H NMR (400 MHz, DMSO) δ 10.8 (brs, 1H), 8.63 (d, 1H, *J* = 1.8 Hz), 8.33 (d, 1H, *J* = 1.8 Hz), 7.81 (ddd, 2H, *J* = 8.6, 1.1, 0.5 Hz), 7.76 (dd, 1H, *J* = 1.5, 0.5 Hz), 7.64 (dd, 1H, *J* = 8.2, 0.5 Hz), 7.48 (ddd, 2H, *J* = 8.6, 1.2, 0.5 Hz), 7.28-7.39 (m, 2H), 7.24 (dd, 1H, *J* = 1.6, 0.5 Hz), 6.81 (dd, 1H, *J* = 8.2, 1.6 Hz), 5.14 (s, 2H). MS (ESI)

m/z: 514.4 $[M + H]^+$ Elem. Anal. calculated for $C_{24}H_{15}F_4N_5O_4$: C, 56.15; H, 2.94; F, 14.80; N, 13.64; O, 12.47Found : C, 56.10; H, 2.85; F, 14.73; N, 13.60; O, 12.41.

CONCLUSION

Based on the results of the 3D QSAR, we described the synthesis of selected novel biphenyl derivatives of benzimidazole in the present work. The synthesized compounds were characterized by 1H NMR, and Mass spectroscopy and the obtained results are showing good agreement with the synthesized structures. Amongst the synthesized compounds screened for their antimycobacterial activity, compounds 6c and 6d showed good potency and some compounds (6e and 6f) are equally active as compared to standard dugs. These results make novel biphenyl derivatives of benzimidazole derivatives, an interesting lead molecule for more synthetic and biological evaluation. It can be concluded that this class of compounds certainly hold great promise towards pursuit to discover novel class of antimycobacterial agents.

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