Current Bioactive Compounds 2017, *13*, 244-258

RESEARCH ARTICLE

Synthesis and Molecular Modeling Studies of Novel 2,4-Disubstituted-1, 5-Diphenyl-1-*H*-Imidazole Derivatives as Potential Anti-Tubercular Agents

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Abstract: ***Background:*** The present discussion reports the synthesis of a series of novel derivatives 2, 4-disubstituted -1, 5 -diphenyl substituted -1-*H*-imidazole derivatives and their molecular modeling studies as antitubercular agents.

Methods: Various substituted aromatic aldehydes (0.01 mol) and anilines (0.01mol) reacted in the presence of methanol and glacial acetic acid into Schiff bases. Schiff bases were treated with TOSMIC (0.01 mol), dioxane, methanol and K2CO3 to give various key intermediates such as 1, 5- diphenyl substituted -1 -H- imidazole derivatives. 1, 5- diphenyl substituted -1 -H- imidazole derivatives which are utilized to develop further derivatives.

Results: The synthesized derivatives were characterized using IR, 1HNMR and Mass spectra. Compounds A-IVn,B-IVe,B-IVf, B-IVg, B-IVj, B-IVk, B-IVm, B-IVn, C-IVa, C-IVc, exhibited the potent antitubercular activity at (1.6 µg/ml to 100 µg/ml) concentration. The results indicated that compounds containing diphenyl substitution with 2–Fluro, 3-Nitro, 2-Chloro, 4-Bromo, 3-Hydroxy,4-Methoxy,4-Nitro, 3-Chloro, 4-dimethylamino and 2,4-dinitro showed potent anti-tubercular activity. QSAR analysis revealed the importance of electronic and steric parameters in anti-tubercular potential of imidazole derivatives.

Conclusion: Developed Imidazole derivatives showed promising anti-tubercular activity, further development of imidazole derivatives using QSAR and pharmacophore modelling will result in the development of potent anti-tubercular derivatives in the future.

ARTICLE HISTORY

Received: April 26, 2016
Revised: July 25, 2016
Accepted: July 26, 2016

DOI:
10.2174/1573407212666160804122247

Keywords: Imidazole, schiffs base, TOSMIC, antitubercular, QSAR, pharmacophore, Microplate Alamar Blue Assay.

1. INTRODUCTION

Tuberculosis remains a leading cause of mortality worldwide, but the number of approved drugs for the TB treatment is very less [1-3]. The current drug therapy for TB is time consuming and complex, which involves various multidrug combinations [4]. Occurrence of the resistant form of tuberculosis resulted in the infective nature of the currently utilized antitubercular drugs. The need for such lengthy treatment is largely because the drugs are relatively ineffective against the persistent form of the disease [5]. Development of multi-drug-resistant (MDR) strains and XDR strains in HIV patients increased death rate significantly [6-8]. Enoyl acyl carrier protein reductase (FabI) was identified as a novel anti-infective target which is involved in the fatty acid synthesis [9, 10]. Inhibition of InhA disrupts the biosynthesis of the mycolic acids that are central constituents of the mycobacterium cell wall [11]. Quantitative Structure activity relationship (QSAR) models are important and effective in describing the structural basis of biological activity. The success of QSAR approach can be explained by the insight offered into the structural determination of chemical properties, and the possibility to estimate the properties of new chemical compounds without the need to synthesize and test them. In the recent years, there has been concerned search for the discovery and development of potent and selective drugs against antitubercular agents. Imidazoles are probably the most well-known heterocyclic compounds; an important feature of a variety of natural and medicinal agents. Imidazole derivatives are important having

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1875-6646/17 $58.00+.00

variety of applications in the field of medicinal chemistry. Imidazole or its derivatives have received significant attention due to their diverse range of biological properties such as they are well-known analgesics [12] anti-inflammatory [13-16] antiparasitic [17] platelet aggregation inhibitors [18] tuberculostatic [19] anticancer [20] and anticonvulsant [21, 22]. Current manuscript deals with the development of imidazole derivatives as antitubercular agents with potential inhibitory action on NADH-dependent enoyl-ACP reductase of mycobacterium tuberculosis. 40 different imidazole derivatives were synthesized and screened for their antitubercular activity; and two different 3D QSAR models were generated for critical analysis of structural requirement of imidazole derivatives to act as antitubercular agents.

2. EXPERIMENTAL

2.1. Chemistry

The synthetic chemistry involved conversion of various substituted aromatic aldehydes (0.01 mol) and anilines (0.01mol) (Tables **1-3**) in the presence of methanol and GAA into Schiffs bases. Schiffs bases were treated with TOSMIC (0.01 mol), dioxane, methanol and K_2CO_3 to give various key intermediates such as 1, 5- diphenyl substituted -1 -H- imidazole derivatives. 1, 5- diphenyl substituted -1 -H- imidazole derivatives were treated with nitric acid and bromine and glacial acetic acid to give 2-bromo -4-nitro - 1, 5- diphenyl substituted -1 H- imidazole derivatives (A-IVa-o) (Scheme **A**). Then, 1, 5, diphenyl 1-*H*-imidazole was further treated with concentrated sulfuric acid and N-chlorosuccinamide to give 2-chloro 4-sulfonyl 1, 5 diphenyl substituted 1 -*H*- imidazole derivatives (B-IVa-o) (Scheme **B**). 1, 5 diphenyl 1-*H*-imidazole was further treated with DMSO, Sulfoxide and iodine and potassium iodide to give 2-iodo 4-hydroxy methyl, 1, 5 diphenyl substituted 1 -*H*- imidazole derivatives (C-IVa-j) (Scheme **C**).

Imidazoles are heterocyclic compounds containing five membered planar rings. It is soluble in water and other polar solvents. They are amphoteric and therefore can function as both acid and base. It also possesses weakly acidic property. Imidazole derivatives are reported to posses wide spectrum of activities. Imidazole is the main structural component of amino acid histidine, purine histamine vitamin B12 and biotin.

2.2. Materials and Methods

All the reagents, chemicals and solvents were purchased from S.D. Fine Chemicals, Sigma Aldrich and Spectrochem Mumbai. Melting points were determined by open tube capillary method. Completion of reaction was determined by single spotted TLC. ^{1}HNMR (400MHz) Spectra were recorded on advanced spectrophotometer using $CDCl_3$ and DMSO solvent. The chemical schiffs were reported in ppm downfield zero and coupling constant was reported in hertz (Hz). Tetramethyl methyl silane was used as an internal standard. IR spectra (in KBr) were recorded with Brucker spectrophotometer. Molecular wt. was determined by Mass spectra. All the bacterial strains were obtained from MTCC and Gene Bank, Chandigad, India.

2.3. Experimental

2.3.1. Scheme A

2.3.1.1. Synthesis of Schiffs Base [23] (A-Ia-o) [I]

Substituted aniline (0.01 mol) and aryl aldehyde (0.01 mol) were refluxed for 3-4 hr in 50 ml methanol in the presence of catalytic amount of (3 to 4 drops) glacial acetic acid. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. Reaction mixture was poured over crushed ice; precipitate formed was filtered and dried. The structure was confirmed by spectral and analytical data.

2.3.1.2. Synthesis of 1, 5 diphenyl substituted -1-H-imidazole derivatives base [24] (A-IIa-o) [II]

A mixture of compound [I] (0.01 mol) schiffs base, tosylmethyl isocyanides [TOSMIC], (0.01 mol) K_2CO_3 (0.01 mol), 30 ml methanol and 40 ml dioxane was refluxed for 3-4 hrs. Reaction mixture was filtered and the filtrate obtained was evaporated under reduced pressure to give residual mass. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. The latter was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO4 and solvent was evaporated under reduced pressure to give crude product, which was recrystallized by suitable solvent, filtered and dried. The structure was confirmed by spectral and analytical data.

2.3.1.3. Step 3: Synthesis of 4-Nitro 1, 5 diphenyl- substituted -1H –imidazole-derivatives [25] (A-IIIa-o) [III]

A mixture of compound (II) (0.01 mol) treated with 4 ml of concentrated sulfuric acid and drop by drop 4 ml nitric acid was added and allowed to stand for 10 minutes. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. Reaction mixture was poured over crushed ice; precipitate formed was filtered and dried. The structure was confirmed by spectral and analytical data.

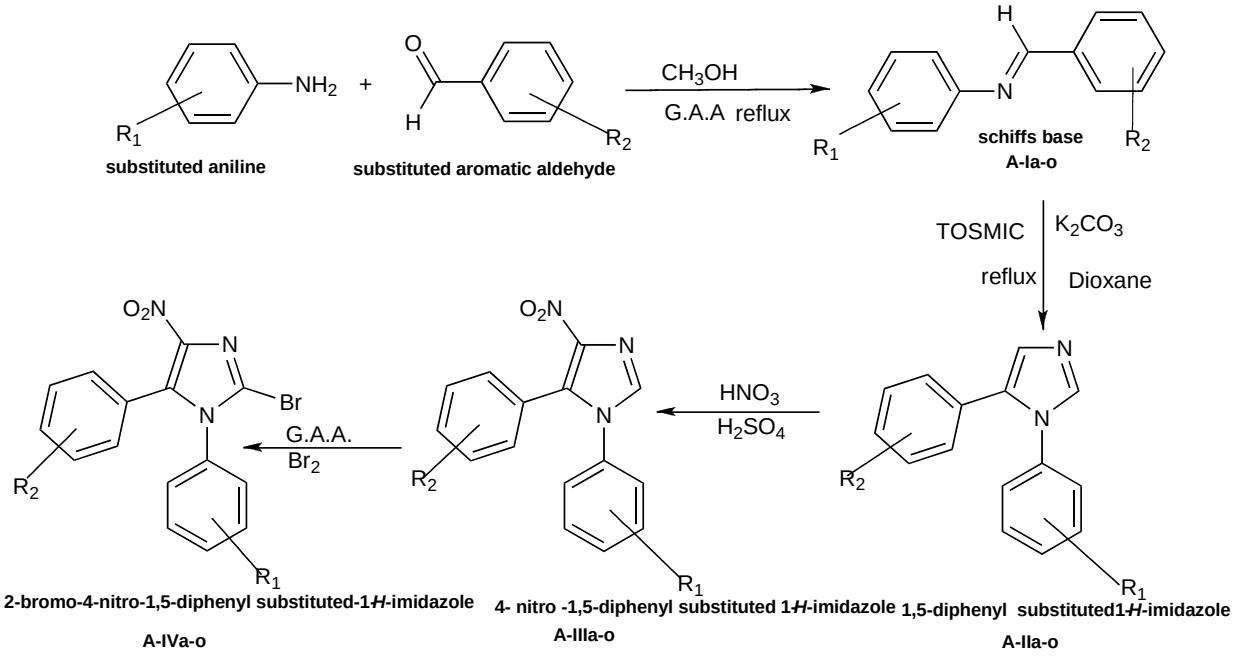
2.3.1.4. Step 4: Synthesis of 2 -Bromo 4-Nitro 1, 5 diphenyl- substituted -1H –imidazole derivatives [26, 27] (A-IVa-o) [IV]

Mixture of compound [III] (0.01 mol) treated with 10-15 ml of glacial acetic acid and 3-4 ml of liquid bromine was allowed to stand for 15-20 minutes. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. Reaction mixture was poured over crushed ice; precipitate formed was filtered and dried. The structure was confirmed by spectral and analytical data.

2.3.2. Scheme B

2.3.2.1. Synthesis of Schiffs base (B-Ia -o) [I]

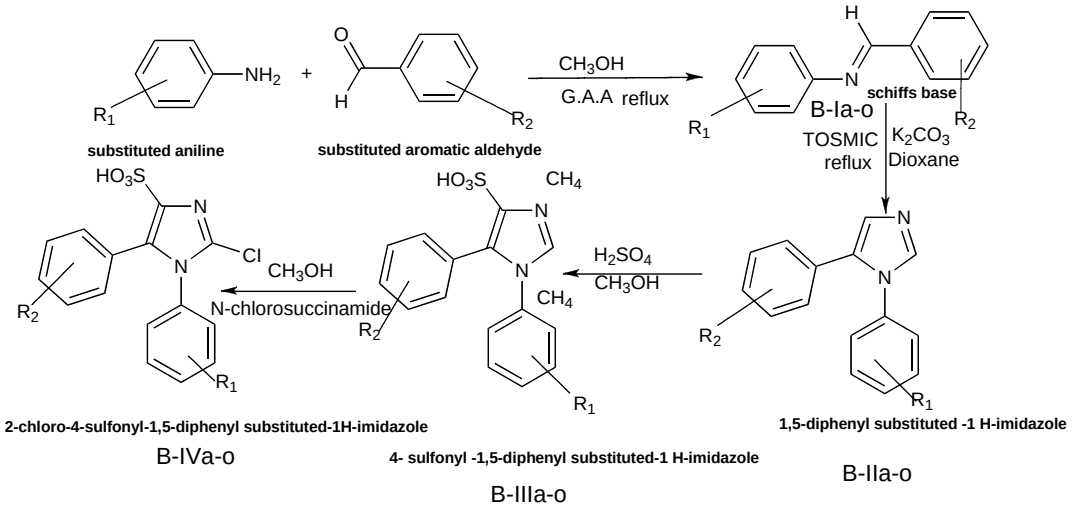
Substituted aniline (0.01 mol) and aryl aldehyde (0.01 mol) were refluxed for 3-4 hours in 50 ml methanol in the presence of catalytic amount of (3 to 4 drops) glacial acetic acid. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction.



Scheme A. Synthetic reaction pathway of 2- bromo-4-nitro-1,5-diphenyl substitutd 1-*H*-imidazole moiety.

Table 1. R_1, R_2 substitutions and physicochemical data (A-IVa to IVo).

Compound No.	R_1 (Anilines)	R_2 (Aldehydes)	Molecular Formula	Molecular Weight	Melting Point (°C)	% Yield
A-IVa	4-F	P-Dimethyl amino	$C_{17}H_{16}BrFN_4O_2$	407.2	112-114	46
A-IVb	2,4-di nitro	2- NO_2	$C_{15}H_7BrN_6O_8$	479.1	106-108	44
A-IVc	2,4-di nitro	4-Cl	$C_{15}H_9BrClN_5O_6$	470.6	118-120	47
A-IVd	4-NO_2	P-Dimethylamino	$C_{17}H_{16}BrN_5O_4$	434.2	113-115	51
A-IVe	Aniline	2-NO_2	$C_{15}H_{12}BrN_3O_3$	362.1	125-127	53
A-IVf	2,4-di nitro	4-OH	$C_{15}H_{10}BrN_5O_7$	452.1	102-104	62
A-IVg	2,4-di nitro	4- NO_2	$C_{15}H_7BrN_6O_8$	479.1	121-123	56
A-IVh	2-NO_2	4-OH	$C_{15}H_9BrN_4O_5$	432.2	109-111	54
A-IVi	2,4-di nitro	p-Dimethylamino	$C_{15}H_{14}BrN_5O_4$	432.1	111-113	58
A-IVj	2-F	2- NO_2	$C_{15}H_8BrFN_4O_4$	407.1	117-119	60
A-IVk	2,4-dinitro	Benzaldehyde	$C_{15}H_{10}BrN_5O_6$	436.1	114-16	62
A-IVl	Aniline	4-OH	$C_{15}H_{12}BrN_3O_3$	362.1	113-15	63
A-IVm	2-F	4-Cl	$C_{15}H_{10}BrClFN_3O_2$	398.6	103-5	53
A-IVn	3-Cl	4-OH	$C_{15}H_{11}BrClN_3O_3$	396.6	102-105	64
A-IVo	4-Br	2-Cl	$C_{15}H_{10}Br_2ClN_3O_2$	459.5	107-109	58



Scheme B. Synthetic reaction pathway of 2-chloro -4-sulfonyl-1,5-diphenyl substituted-1H-imidazole derivatives.

Table 2. R_1, R_2 substitutions and physicochemical data (B-IVa to IVo).

Compound No.	R_1 (Anilines)	R_2 (Aldehydes)	Molecular Formula	Molecular Weight	Melting Point (°C)	% Yield
B-IVa	4-Cl	P-Dimethylamino	$C_{17}H_{15}Cl_2N_3O_3S$	412.2	102-104	56
B-IVb	4-Cl	4- NO_2	$C_{15}H_9Cl_2N_3O_5S$	414.2	111-114	58
B-IVc	4-Cl	4-Cl	$C_{15}H_9Cl_3N_2O_3S$	403.6	98-100	57
B-IVd	3- NO_2	2-Cl	$C_{15}H_9Cl_2N_3O_5S$	414.2	97-99	60
B-IVe	2-F	3-NO_2	$C_{15}H_9ClFN_3O_5S$	397.7	113-115	49
B-IVf	2-Cl	2-NO_2	$C_{15}H_9Cl_2N_3O_5S$	414.2	117-120	60
B-IVg	4- NO_2	2-Cl	$C_{15}H_9Cl_2N_3O_5S$	414.2	122-124	55
B-IVh	4-NO_2	4-OCH_3	$C_{16}H_{12}ClN_3O_6S$	409.8	125-126	62
B-IVi	2-Br	3-OH	$C_{15}H_{10}BrClN_2O_4S$	429.6	115-117	50
B-IVj	4-NO_2	4-OH	$C_{15}H_{10}ClN_3O_6S$	395.7	119-120	51
B-IVk	3- NO_2	4- OCH_3	$C_{16}H_{12}ClN_3O_6S$	409.8	112-115	53
B-IVl	2-Br	4-OH	$C_{15}H_{10}BrClN_2O_4S$	429.6	115-118	56
B-IVm	3-Cl	p-dimethylamino	$C_{18}H_{16}Cl_2N_2O_3S$	411.3	132-134	48
B-IVn	4-Br	3-OH	$C_{15}H_{10}BrClN_2O_4S$	429.6	135-136	60
B-IVo	2-F	4- NO_2	$C_{15}H_9ClFN_3O_5S$	397.7	139-142	50

Reaction mixture was poured over crushed ice; precipitate formed was filtered and dried. The structure was confirmed by spectral and analytical data.

2.3.2.2. Synthesis of 1, 5-diphenyl substituted -1-H-imidazole derivatives (B-IIa -o) [II]

A mixture of compound (B-IIa-o) (0.01 mol) schiffs base, tosylmethyl isocyanides [TOSMIC], (0.01 mol) K_2CO_3 (0.01 mol), 30 ml methanol and 40 ml dioxane was refluxed for 3-4 hours. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. Reaction mixture was filtered and filtrate obtained was evaporated under reduced pressure to give residual mass. The latter was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO4 and solvent was evaporated under reduced pressure to give crude product, which was recrystallized by suitable solvent, filtered and dried. The structure was confirmed by spectral and analytical data.

2.3.2.3. Synthesis of 4-Sulfonyl1, 5-diphenyl substituted -1H-imidzole derivatives [28] (B-IIIa-o) [III]

A mixture of compound (B-IIa-o) (0.01 mol) and 3-4 ml of concentrated sulfuric acid was boiled for 5 minute and cooled in ice cold water. Lower layer of crude sulphonic acid

was solidified and the upper layer was discarded. Then, 10 ml of concentrated hydrochloric acid was added to the mixture and heated gently until clear solution was obtained. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. Then, solution was poured into ice cold water which gave precipitate which was filtered, dried and recrystallized by suitable solvent. The structure was confirmed by spectral and analytical data.

2.3.2.4. Synthesis of 2-Chloro -4-Sulfonyl1, 5-diphenyl substituted -1H-imidazole derivatives [29] (B-IVa-o) [IV]

A mixture of compound (B-IIIa-o) (0.01 mol), N-chlorosuccinamide, (0.01 mol), methanol (30 ml) and dioxane (10 ml) was stirred at 65°C for 24 hour. Reaction mixture was added into (10 ml) water and extracted with chloroform (45 ml). Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. The organic layer was washed with water, dried and recrystallized by suitable solvent. The structure was confirmed by spectral and analytical data.

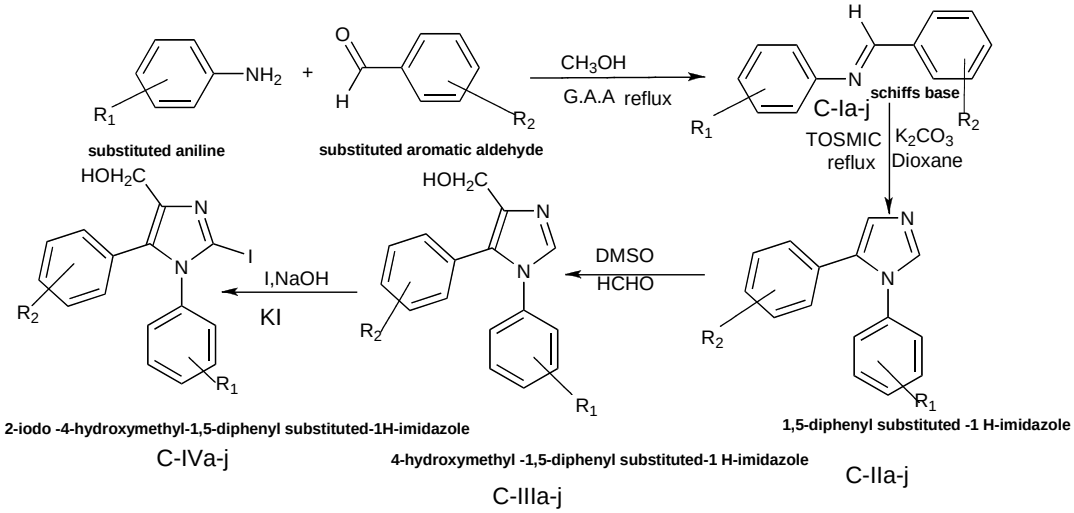
2.3.3. Scheme C

2.3.3.1. Synthesis of Schiff base – (C-Ia-j) [I]

Substituted aniline (0.01 mol) and aryl aldehyde (0.01 mol) were refluxed for 3-4 hr. In 50 ml methanol in the presence of catalytic amount of (3 to 4 drops) glacial acetic acid. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. Reaction mixture was poured over crushed ice; precipitate formed was filtered and dried. The structure was confirmed by spectral and analytical data.

2.3.3.2. Synthesis of 1, 5 diphenyl substituted -1-H-imidazole derivatives base (C-IIa-j) [II]

A mixture of compound [C-IIa -j] (0.01 mol) schiffs base, tosylmethyl isocyanides [TOSMIC], (0.01 mol) K_2CO_3



Scheme C. Synthetic reaction pathway of 2-Iodo -4-hydroxymethyl -1,5-diphenyl substituted-1H-imidazole.

Table 3. R_1, R_2 substitutions and physicochemical data (C-IVa to IVj).

Compound No.	R_1 (Anilines)	R_2 (Aldehydes)	Molecular Formula	Molecular Weight	Melting Point (°C)	% Yield
C-IVa	3-Cl	3-OH	$C_{16}H_{12}ClIN_2O_2$	426.6	112-115	57
C-IVb	2,4dinitro	2- Cl	$C_{16}H_{10}ClIN_4O_5$	500.6	110-113	45
C-IVc	2-NO_2	3-OH	$C_{16}H_{12}IN_3O_4$	437.1	121-123	50
C-IVd	2-F	4-Br	$C_{16}H_{11}BrFIN_2O$	473.0	124-126	53
C-IVe	4-NO_2	3-OH	$C_{16}H_{12}IN_3O_4$	437.1	117-119	49
C-IVf	2,4-dinitro	3-OH	$C_{16}H_{11}IN_4O_6$	482.1	122-124	60
C-IVg	2-NO_2	4-Br	$C_{16}H_{11}BrIN_3O_3$	500.0	101-103	62
C-IVh	4-NO_2	4--Br	$C_{16}H_{11}BrIN_3O_3$	500.0	107-109	55
C-IVi	4-F	4-Br	$C_{16}H_{11}BrFIN_2O$	473.0	106-108	48
C-IVj	3-NO_2	3-OH	$C_{16}H_{12}IN_3O_4$	437.1	111-113	54

(0.01 mol), 30 ml methanol and 40 ml dioxane was refluxed for 3-4 hours. Reaction mixture was filtered and filtrate obtained was evaporated under reduced pressure to give residual mass. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. The latter was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over Na_2SO4 and solvent was evaporated under reduced pressure to give crude product, which was recrystallized by a suitable solvent, filtered and dried. The structure was confirmed by spectral and analytical data.

2.3.3.3. Synthesis of 4-Hydroxymethyl -1,5-diphenyl -1H-imidazole derivatives [30] (C-IIIa-j) [III]

A mixture of compound (C-IIa-j) (0.01 mol), formaldehyde (10 ml) and 6 ml DMSO (dimethyl sulfoxide) was heated at 120-130^{o}C for 3 hr. In alkaline medium by using 3% sodium hydroxide solution. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. Then, reaction mixture was poured into ice cold water which give precipitate which was filtered dried and recrystallized by a suitable solvent. The structure was confirmed by spectral and analytical data.

2.3.3.4. Synthesis of 4-Hydroxymethyl 2-Iodo-1,5-diphenyl -1H-imidazole derivatives [31] (C-IVa-j) [IV]

A mixture of compound (C-IIIa-j) (0.01 mol) and iodine (0.01 mol) in 10% aqueous potassium iodide (30 ml) was added drop wise to stirred solution of 2 M sodium hydroxide (30 ml) at ambient temperature and the resulting mixture was stirred overnight and 25% aqueous acetic acid was added until mixture was neutral to give precipitate which was filtered, washed with water and air dried. Progress of reaction was monitored by TLC (thin layer chromatography) indicating the completion of reaction. The precipitate was filtered washed with water, dried and recrystallized by a suitable solvent. The structure was confirmed by spectral and analytical data.

2.3.4. Scheme-A

2.3.4.1. Analytical Data

*2.3.4.1.1. 4-[2-bromo-1-(4-fluorophenyl)-4-nitro-4,5-dihydro-1*H-*imidazol-5-yl]*-N,N-*dimethylaniline (A-IVa)*

IR- 3002 C-H (Aromatic Str.) 1747C=C (Aromatic), 1528 NO_2, 1472 C-C (Aromatic), 1266 C=N, 1109 C-N, 925 C-F, 763 C-Br, 685 C-H (Aromatic Deformation).

^{1}HNMR- 6.718 (s, 2H), 6.441(s, 2H), 6.230 (s, 2H), 6.142(s, 2H), 3.759- 3.710 (d, 1H), 3.642-3.563 (d, 1H), 1.861 (s, 6H).

MASS- 408.2 (M+1), 407.2, 363, 359, 312, 287, 239, 144, 120, 78, 66.

*2.3.4.1.2. 2-2-bromo-5-(2,4-dinitrophenyl)-4-nitro-1-(2-nitrophenyl)-1*H-*imidazole (A-IVb)*

IR- 3083 C-H (Aromatic Str.) 1617C=C (Aromatic), 1578 NO_2, 1497 C-C (Aromatic), 1326 C=N, 1121 C-N, 908 C-F, 733 C-Br, 694 C-H (Aromatic Deformation).

^{1}HNMR- 6.74-6.71 (d, 1H), 6.60-6.58 (d, 1H), 6.42 (s, 1H), 6.18 (s, 1H), 6.01 (s, 1H), 5.96 (s, 1H), 5.62 (s, 1H), 5.44 (s, 1H).

MASS- 408.1 (M+1), 407.1, 359, 311, 283, 264, 235, 124, 78, 66.

*2.3.4.1.3. 2-bromo-5-(4-chlorophenyl)-1-(2, 4-dinitrophenyl)-4-nitro-4, 5-dihydro-1*H-*imidazole (A-IVc)*

IR- 3123 C-H (Aromatic Str.)1716C=C (Aromatic), 1564 NO_2, 1428 C-C (Aromatic), 1267 C=N, 1130 C-N, 925 C-F, 719 C-Br, 686 C-H (Aromatic Deformation).

^{1}HNMR- 7.011 (s, 1H), 6.854(s, 1H), 6.623(s, 1H), 6.378(s, 1H), 6.127 (s, 1H), 5.786(s, 1H), 4.201-4.059 (d, 1H), 3.921-3.876 (d, 1H), 3.607-3.403(d, 1H).

MASS- 471.5 (M+1), 470.5, 422, 374, 359, 347, 311, 159, 123, 111, 96, 78, 66.

*2.3.4.1.4. 4-[2-bromo-4-nitro-1-(4-nitrophenyl)-4,5-dihydro-1*H-*imidazol-5-yl]*-N,N- *dimethylaniline (A-IVd)*

IR- 2914 C-H (Aromatic Str.), 1602C=C (Aromatic), 1526 NO_2, 1497 C-C (Aromatic), 1316 C=N, 1120 C-N, 728 C-Br, 674 C-H (Aromatic Deformation).

^{1}HNMR- 6.541 (s, 1H), 6.387 (s, 1H), 6.189 (s, 1H), 5.979 (s, 1H), 4.488-4.421 (d, 1H), 4.310-4.185 (d, 1H), 1.184 (s, 6H).

MASS- 435.1 (M+1), 434.1, 433.1, 390, 386, 314, 312, 122, 120, 78, 66.

*2.3.4.1.5. 4-(2-bromo-4-nitro-1-phenyl-4, 5-dihydro-1*H-*imidazol-5-yl) phenol (A-IVe)*

IR- 3355 O-H, 2916 C-H (Aromatic Str.), 1603 C=C (Aromatic), 1527 NO_2, 1334 C-C (Aromatic), 1414 C=N, 1081 C-N, 828 C-Br, 737 C-H (Aromatic Deformation).

^{1}HNMR- 8.421 (s, 1H), 6.796 (s, 1H), 6.456 (s, 1H), 6.148 (s, 1H), 4.165-4.022 (s, 1H), 3.912-3.816 (s, 1H).

MASS- 363, 362, 361, 345, 314, 284, 269, 191, 143, 93, 78, 66.

*2.3.4.1.6. 4-[2-bromo-1-(2, 4-dinitrophenyl)-4-nitro-4, 5-dihydro-1*H-*imidazol-5-yl] phenol (A-IVf)*

IR- 3370 O-H, 2916 C-H (Aromatic Str.), 1616 C=C (Aromatic), 1532 NO_2, 1455 C-C (Aromatic), 1334 C=N, 1113 C-N, 848 C-Br.

^{1}HNMR- 8.604 (s, 1H, OH), 7.241-7.164 (d, 1H), 6.775 (s, 1H), 6.599 (s, 1H), 6.403 (s, 1H), 6.211 (s, 1H), 5.221-5.163 (d, 1H), 4.965-4.891 (d, 1H).

MASS- 452 (M+1), 451, 435, 404, 387, 356, 285, 167, 96, 93, 78, 66.

*2.3.4.1.7. 2-bromo-1-(2, 4-dinitrophenyl)-4-nitro-5-(4-nitrophenyl)-1*H-*imidazole (A-IVg)*

IR- 3076 C-H (Aromatic Str.), 1606 C=C (Aromatic), 1581 NO_2, 1508 C-C (Aromatic), 1330 C=N, 1116 C-N, 688 C-H (Aromatic Deformation).

^{1}HNMR- 7.410-7.254 (d, 1H), 6.887 (s, 1H), 6.606-6.542 (d, 1H), 6.413-6.389 (d, 1H), 5.911 (s, 1H), 5.743 (s, 1H).

MASS- 479, 431,357, 335, 179, 156, 122, 96, 78, 66.

*2.3.4.1.8. 4-[2-bromo-4-nitro-1-(2-nitrophenyl)-1*H-*imidazol-5-yl] phenol (A-IVh)*

IR- 3365 O-H, 2915 C-H (Aromatic Str.), 1618 C=C (Aromatic), 1530 NO_2, 1460 C-C (Aromatic), 1339 C=N, 1113 C-N, 718 C-Br.

^{1}HNMR- 9.142 (s, 1H), 7.380-7.204 (s, 1H), 6.912 (s, 1H), 6.799 (s, 1H), 6.602 (s, 1H), 6.335 (s, 1H), 6.176 (s, 1H).

MASS- 479 (M+1), 478, 414, 366, 340, 122, 96, 78, 66.

*2.3.4.1.9. 4-[2-bromo-1-(2, 4-dinitrophenyl)-1*H-*imidazol-5-yl]-*N, N-*dimethylaniline (A-IVi)*

IR- 2883 C-H (Aromatic Str.), 1594 C=C (Aromatic), 1515 NO_2, 1465 C-C (Aromatic), 1308 C=N, 1059 C-N, 722 C-Br, 638 C-H (Aromatic Deformation).

^{1}HNMR- 6.784-6.686 (d, 1H), 6.501 (s, 1H), 6.216 (s, 1H), 6.087 (s, 1H), 5.823 (s, 1H), 1.531 (s, 1H).

MASS- 405 (M+1), 404, 361, 283, 167, 116, 78, 66.

*2.3.4.1.10. 2-bromo-1-(2-fluorophenyl)-4-nitro-5-(2-nitrophenyl)-1*H-*imidazole (A-IVj)*

IR- 2914 C-H (Aromatic Str.), 1558 C=C (Aromatic), 1524 NO_2, 1463 C-C (Aromatic), 1337 C=N, 1117 C-N, 877 C-F, 737 C-Br, 720 C-H (Aromatic Deformation).

^{1}HNMR- 6.841-6.795 (d, 1H), 6.712-6.620 (d, 1H), 6.535 (s, 1H), 6.378 (s, 1H), 6.204 (s, 1H), 6.007 (s, 1H), 5.809 (s, 1H).

MASS- 408 (M+1), 407, 388, 359, 310, 281, 264, 262, 124, 95, 78, 66.

*2.3.4.1.11. 2-bromo-1-(2,4-dinitrophenyl)-4-nitro-5-phenyl-4, 5-dihydro-1*H-*imidazole (A-IVk)*

IR- 2848 C-H (Aromatic Str.), 1332 C=C (Aromatic), 1458 NO_2, 1612 C-C (Aromatic), 1251 C=N, 965 C-N, 1063 C-F, 811 C-H (Aromatic Deformation) 681 C-Br.

^{1}HNMR- 8.40 (m, 1H), 7.158-7.633 (m, 5H), 2.847 (m 2H).

MASS- 417 (M+1).

*2.3.4.1.12. 4-(2-bromo-4-nitro-1-phenyl-4,5-dihydro-1*H-*imidazol-5-yl) phenol (A-IVl)*

IR- 2847 C-H (Aromatic Str.), 1335 C=C (Aromatic), 1462 NO_2, 1639 C-C (Aromatic), 1240 C=N, 1172 C-N, 1071 C-F, 811 C-H (Aromatic Deformation) 653 C-Br.

^{1}HNMR- 7.411-7.887 (m, 5H), 7.319-7.064 (m, 4H).

MASS- 362 (M+1).

*2.3.4.1.13. 2-bromo-5-(4-chlorophenyl)-1-(2-fluorophenyl)-4-nitro-4, 5-dihydro-1*H-*imidazole. (A-IVm)*

IR- 2847 C-H (Aromatic Str.), 1341 C=C (Aromatic), 1462 NO_2, 1613 C-C (Aromatic), 1255 C=N, 1153 C-N, 1012 C-F, 853 C-H (Aromatic Deformation) 657 C-Br.

^{1}HNMR- 7.484-7.072 (m, 4H), 7.672-7.504 (m, 4H).

MASS- 401 (M+1).

*2.3.4.1.14. 4-[2-bromo-1-(3-chlorophenyl)-4-nitro-4, 5-dihydro-1*H-*imidazol-5-yl] phenol (A-IVn)*

IR- 3403 O-H str., 2845 C-H (Aromatic Str.), 1389 C=C (Aromatic), 1539 NO_2, 1628 C-C (Aromatic), 1285 C=N, 1156 C-N, 910 C-Cl, 854 C-H (Aromatic Deformation) 744 C-Br.

^{1}HNMR- 9.951 (s, O-H,1H) 8.039 (s, 1H) 7.992-7.894 (m, 4H), 7.288 (s, 1H) 7.160 (s, 1H), 7.032 (s, 1H).

MASS- 397 (M+1).

*2.3.4.1.15. 2-bromo-1-(4-bromophenyl)-5-(2-chlorophenyl)-4-nitro-4,5-dihydro-1*H-*imidazole (A-IVo)*

IR- 2847 C-H (Aromatic Str.), 1388 C=C (Aromatic), 1467 NO_2, 1698 C-C (Aromatic), 1234 C=N, 1135 C-N, 1015 C-Cl, 852 C-H (Aromatic Deformation) 664 C-Br.

^{1}HNMR- 7.489-7.188 (m, 8H).

MASS- 460 (M+1).

2.3.5. Scheme-B

2.3.5.1. Spectral Analysis

*2.3.5.1.1. 2-chloro-1-(4-chlorophenyl)-5-[4-(dimethylamino) phenyl]-1*H-*imidazole-4-sulfonic acid (B-IVa)*

IR- 3038 O-H str., 2847 C-H (Aromatic Str.), 1315 C=C (Aromatic), 1702 C-C (Aromatic), 1224 C=N, 1136 C-N, 1010 C-Cl, 912 S-O, 851 C-H (Aromatic Deformation).

^{1}HNMR- 7.922-7.735 (m, 8H), 4.853-4.609 (m, 3H), 4.399-4.377 (m, 3H).

MASS- 414 (M+1).

*2.3.5.1.2. 2-chloro-1-(4-chlorophenyl)-5-(4-nitrophenyl)-1*H-*imidazole-4-sulfonic acid (B-IVb)*

IR- 3034 O-H str., 2848 C-H (Aromatic Str.), 1314 C=C (Aromatic), 1338 NO_2, 1593 C-C (Aromatic), 1235 C=N, 1182 C-N, 1013 C-Cl, 917 S-O, 52 C-H (Aromatic Deformation).

^{1}HNMR- 11.034 (s, 1H), 8.178-7.086 (m, 7H), 5.752(d, 1H).

MASS- 414 (M+1).

*2.3.5.1.3. 2-chloro-1,5-bis(4-chlorophenyl)-1*H-*imidazole-4-sulfonic acid (B-IVc)*

IR- 3363 O-H str., 2847 C-H (Aromatic Str.), 1493 C=C (Aromatic), 1516 C-C (Aromatic), 1241 C=N, 1180 C-N, 1017 C-Cl, 917 S-O, 813 C-H (Aromatic Deformation).

^{1}HNMR- 11.083 (s, 1H), 8.767-5.801 (m, 8H).

MASS- 327 (M+1).

*2.3.5.1.4. 2-chloro-5-(2-chlorophenyl)-1-(3-nitrophenyl)-1*H-*imidazole-4-sulfonic acids (B-IVd)*

IR- 2916 O-H str., 2848 C-H (Aromatic Str.), 1464 C=C (Aromatic), 1527 C-C (Aromatic), 1343 NO_2, 1236 C=N, 1184C-N, 917 S-O, 803 C-H (Aromatic Deformation).

^{1}HNMR- 11.057 (s, 1H), 8.767-5.801 (m, 8H).

MASS- 424 (M+1).

2.3.5.1.5. 2-chloro-1-(2-fluorophenyl)-5-(3-nitrophenyl)-1H-imidazole-4-sulfonic acid (B-IVe)

IR- 3046 O-H str., 2846 C-H (Aromatic Str.), 1464 C=C (Aromatic), 1519 C-C (Aromatic), 1343 NO_2, 1233 C=N, 1182 C-N, 917 S-O, 851 C-F, 807 C-H (Aromatic Deformation).

^{1}HNMR- 11.041 (s, 1H), 7.739-7.090 (m, 8H).

MASS- 398 (M+1).

*2.3.5.1.6. 2-chloro-1-(4-chlorophenyl)-5-(2-nitrophenyl)-1*H-*imidazole-4-sulfonic acids (B-IVf)*

IR- 3032 O-H str., 2846 C-H (Aromatic Str.), 1489 C=C (Aromatic), 1523 C-C (Aromatic), 1401 NO_2, 1230 C=N, 1186 C-N, 1081 S-O, 1036 C-Cl, 806 C-H (Aromatic Deformation).

^{1}HNMR- 7.774-7.397 (m, 8H).

MASS- 414 (M+1).

*2.3.5.1.7. 2-chloro-5-(2-chlorophenyl)-1-(4-nitrophenyl)-1*H-*imidazole-4-sulfonic acid.(B-IVg)*

IR- 2963 O-H str., 2848 C-H (Aromatic Str.), 1507 C=C (Aromatic), 1576 C-C (Aromatic), 1417 NO_2, 1235 C=N, 184 C-N, 924 S-O, 1184 C-Cl, 858 C-H (Aromatic Deformation).

^{1}HNMR- 11.061 (s, 1H), 8.859-7.968 (m, 8H).

MASS- 414 (M+1).

*2.3.5.1.8. 2-chloro-5-(4-methoxyphenyl)-1-(4-nitrophenyl)-1*H-*imidazole-4-sulfonic acid (B-IVh)*

IR- 2916 O-H str., 2848 C-H (Aromatic Str.), 1507 C=C (Aromatic), 1464 C-C (Aromatic), 1295 NO_2, 1235 C=N, 1183 C-N, 1118 S-O, 852 C-H (Aromatic Deformation).

^{1}HNMR- NMR- 10.235 (s,1H), (m,6.843-7.996) (8),2.201 (s,3H).

MASS- 410 (M+1).

*2.3.5.1.9. 1-(2-bromophenyl)-2-chloro-5-(3-hydroxyphenyl)-1*H-*imidazole-4-sulfonic acid. (B-IVi)*

IR- 2959 O-H str., 2848 C-H (Aromatic Str.), 1704 C=C (Aromatic), 1528 C-C (Aromatic), 1222 C=N, 1184 C-N, 1017 S-O, 917 C-Cl, 857 C-H (Aromatic Deformation).

^{1}HNMR- 9.505 (s, 1H), 8.793 (s, 1H).

MASS- 414 (M+1).

*2.3.5.1.10. 2-chloro-5-(4-hydroxyphenyl)-1-(4-nitrophenyl)-1*H-*imidazole-4-sulfonic acid (B-IVj)*

IR- 2954 O-H str., 2849 C-H (Aromatic Str.), 1674 C=C (Aromatic), 1519 C-C (Aromatic), 1462 NO_2, 1242 C=N, 1187 C-N, 1019 S-O, 1107 C-Cl, 851 C-H (Aromatic Deformation).

^{1}HNMR- 11.059 (s, 1H), 8.855-7.325 (m, 8H).

MASS- 396 (M+1).

*2.3.5.1.11. 2-chloro-5-(4-methoxyphenyl)-1-(3-nitrophenyl)-1*H-*imidazole-4-sulfonic acid (B-IVk)*

IR- 2918 O-H str., 2849 C-H (Aromatic Str.), 1674 C=C (Aromatic), 1518 C-C (Aromatic), 1475 NO_2, 1234 C=N, 1187 C-N, 1077 S-O, 1135 C-Cl, 851 C-H (Aromatic Deformation).

^{1}HNMR- 11.064 (s, 1H), 8.230-7.499 (m, 8H).

MASS- 410 (M+1).

*2.3.5.1.12. 1-(2-bromophenyl)-2-chloro-5-(4-hydroxyphenyl)-1*H-*imidazole-4-sulfonic acid (B-IVI)*

IR- 3068 O-H str., 2926 C-H (Aromatic Str.), 1612 C=C (Aromatic), 1527 C-C (Aromatic), 1276 C=N, 1085 C-N, 983 S-O, 1157 C-Cl, 851 C-H (Aromatic Deformation).

^{1}HNMR- 11.050 (s, 1H), 7.947-7.463 (m, 8H).

MASS- 430 (M+1).

*2.3.5.1.13. 2-chloro-1-(3-chlorophenyl)-5-[4-(propan-2-yl) phenyl]-1*H-*imidazole-4-sulfonic acid (B-IVm)*

IR- 3069 O-H str., 2924 C-H (Aromatic Str.), 1603 C=C (Aromatic), 1524 C-C (Aromatic), 1281 C=N, 1081 C-N, 987 S-O, 1153 C-Cl, 857 C-H (Aromatic Deformation).

^{1}HNMR- 7.873-7.441 (m, 8H), 3.940 (s, 1H), 2.582-2.518 (s, 3H), 2.324-2.255 (s, 3H).

MASS- 379 (M+1).

*2.3.5.1.14. 1-(4-bromophenyl)-2-chloro-5-(3-hydroxyphenyl)-1*H-*imidazole-4-sulfonic acid (B-IVn)*

IR- 3069 O-H str., 2924 C-H (Aromatic Str.), 1603 C=C (Aromatic), 1524 C-C (Aromatic), 1281 C=N, 1081 C-N, 987 S-O, 1153 C-Cl, 857 C-H (Aromatic Deformation).

^{1}HNMR- 11.050 (s, 1H), 7.947-7.463 (m, 8H).

MASS- 379 (M+1).

*2.3.5.1.15. 2-chloro-1-(2-fluorophenyl)-5-(4-nitrophenyl)-1*H-*imidazole-4-sulfonic acid (B-IVo)*

IR- 2915 O-H str., 2847 C-H (Aromatic Str.), 1596 C=C (Aromatic), 1514 C-C (Aromatic), 1462 NO_2, 1263 C=N, 1180 C-N, 1013 S-O, 959 C-F, 851 C-H (Aromatic Deformation).

^{1}HNMR- 8.112-7.094 (m, 8H).

MASS- 366 (M+1).

2.3.6. Scheme-C

2.3.6.1. Spectral Analysis

*2.3.6.1.1. 3-[1-(3-chlorophenyl)-4-(hydroxymethyl)-2-iodo-1*H-*imidazol-5-yl]phenol (C-IVa)*

IR- 2915 O-H str., 2847 C-H (Aromatic Str.), 1461 C=C (Aromatic), 1433 C-C (Aromatic), 1246 C=N, 1183 C-N, 1067 C-Cl, 856 C-H (Aromatic Deformation).

^{1}HNMR- 11.064 (s, 1H), 7.486-7.157 (m, 8H), 3.186 (s, 2H).

MASS- 424 (M+1).

*2.3.6.1.2. [5-(2-chlorophenyl)-1-(2,4-dinitrophenyl)-2-iodo-1*H*-imidazol-4-yl]methanol (C-IVb)*

IR- 3050 O-H str., 2847 C-H (Aromatic Str.), 1486 C=C (Aromatic), 1486 NO_2, 1399 C-C (Aromatic), 1230 C=N, 1183 C-N, 1074 C-Cl, 808 C-H (Aromatic Deformation).

^{1}HNMR- 8.829-7.029 (m, 7H), 2.520 (d, 2H).

MASS- 491 (M+1).

*2.3.6.1.3. 3-[4-(hydroxymethyl)-2-iodo-1-(2-nitrophenyl)-1*H*-imidazol-5-yl]phenol (C-IVc)*

IR- 3048 O-H str., 2845 C-H (Aromatic Str.), 1483 C=C (Aromatic), 1480 NO_2, 1397 C-C (Aromatic), 1237 C=N, 1184 C-N, 1068 C-Cl, 816 C-H (Aromatic Deformation).

^{1}HNMR- 10.856 (s, 1H), 10.761 (s, 1H), 7.623-7.159 (m, 8H), 2.540 (s, 2H).

MASS- 428 (M+1).

*2.3.6.1.4. [5-(4-bromophenyl)-1-(2-fluorophenyl)-2-iodo-1*H*-imidazol-4-yl] methanol (C-IVd)*

IR- 3423 O-H str., 2846 C-H (Aromatic Str.), 1486 C=C (Aromatic), 1413 C-C (Aromatic), 1282 C-F, 1246 C=N, 1185 C-N, 1069 C-Cl, 844 C-H (Aromatic Deformation).

^{1}HNMR- 10.001 (s, 1H), 7.281-6.991 (m, 8H), 2.570 (s, 1H).

MASS- 487 (M+1).

*2.3.6.1.5. 3-[4-(hydroxymethyl)-2-iodo-1-(4-nitrophenyl)-1*H*-imidazol-5-yl]phenol (C-IVe)*

IR- 2912 O-H str., 2845 C-H (Aromatic Str.), 1468 C=C (Aromatic), 1439 C-C (Aromatic), 1293 C=N, 1174 C-N, 832 C-H (Aromatic Deformation).

^{1}HNMR- 10.546 (d, 2H), 6.032-7.726 (m, 8H), 2.570 (s, 2H).

MASS- 448 (M+1).

*2.3.6.1.6. 3-[4-(hydroxymethyl)-2-iodo-1-(4-nitrophenyl)-1*H*-imidazol-5-yl]phenol (C-IVf)*

IR- 2915 O-H str., 2847 C-H (Aromatic Str.), 1516 C=C (Aromatic), 1415 C-C (Aromatic), 1285 C=N, 1128 C-N, 801 C-H (Aromatic Deformation).

^{1}HNMR- 10.389 (d, 2H), 7.343-7.084 (m, 7H), 2.829 (s, 2H).

MASS- 491 (M+1).

*2.3.6.1.7. [5-(4-bromophenyl)-2-iodo-1-(2-nitrophenyl)-1*H*-imidazol-4-yl]methanol (C-IVg)*

IR- 3014 O-H str., 2858 C-H (Aromatic Str.), 1523 C=C (Aromatic), 1452 C-C (Aromatic), 1279 C=N, 1115 C-N, 803 C-H (Aromatic Deformation), 749 C-Br.

^{1}HNMR- 10.052 (s, 1H), 6.840-7.144 (m, 8H), 2.686 (s, 2H).

MASS- 501 (M+1).

*2.3.6.1.8. [5-(4-bromophenyl)-2-iodo-1-(4-nitrophenyl)-1*H*-imidazol-4-yl]methanol (C-IVh)*

IR- 2956 O-H str., 2848 C-H (Aromatic Str.), 1494 C=C (Aromatic), 1403 C-C (Aromatic), 1303 C=N, 1181 C-N, 811 C-H (Aromatic Deformation), 744 C-Br.

^{1}HNMR- 10.498 (s, 1H), 7.452-7.293 (m, 8H), 2.414-2.369 (m, 2H).

MASS- 501 (M+1).

*2.3.6.1.9. [5-(4-bromophenyl)-1-(4-fluorophenyl)-2-iodo-1*H*-imidazol-4-yl]methanol (C-IVi)*

IR- 2917 O-H str., 2849 C-H (Aromatic Str.), 1504 C=C (Aromatic), 1408 C-C (Aromatic), 1215 C=N, 1150 C-F, 1066 C-N, 809 C-H (Aromatic Deformation), 648 C-Br.

^{1}HNMR- 10.816 (s, 1H), 7.260-7.083 (m, 8H), 2.518-2.355 (m, 2H).

MASS- 474 (M+1).

*2.3.6.1.10. 3-[4-(hydroxymethyl)-2-iodo-1-(3-nitrophenyl)-1*H*-imidazol-5-yl]phenol (C-IVj)*

IR- 2919 O-H str., 2839 C-H (Aromatic Str.), 1514 C=C (Aromatic), 1406 C-C (Aromatic), 1218 C=N, 1142 C-F, 1050 C-N, 812 C-H (Aromatic Deformation).

^{1}HNMR- 10.483 (s, 1H), 7.349-7.071 (m, 8H), 2.473-2.333 (m, 2H).

MASS- 438 (M+1).

2.4. QSAR Analysis

2.4.1. Ligand Preparation

The structure of imidazole derivatives was used as the template to build the molecules in the builder module on Vlife MDS 4.4 software. The ligand geometries were optimized using MMFF94 force field and Gasteiger-Marsili charges.

2.4.2. Molecular Alignment

The template based technique was used for the alignment of the dataset molecules. Template for alignment was selected on the basis of the most active molecule and the alignment of all the molecules is shown in Fig. (**1**).

2.4.3. Descriptor Calculation

Descriptor calculation was carried out using Vlife MDS 4.3. Descriptors are the hydrophilic, steric, and electrostatic interaction energies which were computed at the lattice points.

2.4.4. Data Set

The synthesized molecules were classified into the training and test set using random selection methods. All the molecules were assorted into a training set (26 Molecules) and a test set (14 molecules) using random selection on the basis of chemical and biological diversity.

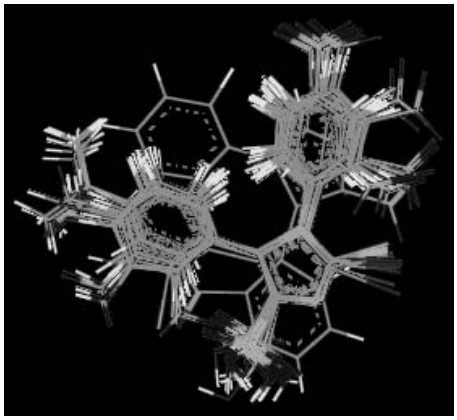


Fig. (1). Alignment of molecules using the most active compound template.

2.4.5. Full Search Multiple Linear Regression Method

By using regression analysis, relationship between independent and dependent variables was determined (3D fields and biological activities, respectively). Models having significant correlation coefficient, q^2 were selected and other developed models were discarded.

2.4.6. Docking Studies

Docking simulations were carried out using Biopredicta module of Vlife MDS 4.3 using crystal structure of NADH-Dependent enoyl-ACP-reductase. (**PDBID:** ***1IDZ***) downloaded from protein database *www.rcsb.org*.

2.4.7. Pharmacophore Modeling

Pharmacophore modeling was also carried out in Vlife MDS 4.3 using Mol sign module. The minimum number of pharmacophore features generated for an alignment is 4 and tolerance is kept at 10 Å.

2.5. *In Vitro* Anti-TB Activity Using Alamar Blue Dye Method [32]

2.5.1. Materials

M. tuberculosis (H37 RV strain ATCC No- 27294. BACTEC radiometric methods, Alamar Blue and deionized water.

2.5.2. Method

The anti-mycobacterial activity of compounds was assessed against M. tuberculosis using Microplate Alamar Blue Assay (MABA) (Table **4**). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on the plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25 µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% Tween 80 were added to the plate and incubated for 24 hrs.

Table 4. Anti tubercular activity by Almar Blue Assay.

Sr. No.	Samples	Activity (MIC_{50})	Predicted Activity (MIC_{50})
1	A-IVa	0.71	0.70
2	A-IVb	0.71	0.75
3	A-IVc	0.71	0.76
4	A-IVd	0.71	0.77
5	A-IVe	0.71	0.70
6	A-IVf	0.71	0.70
7	A-IVg	0.71	0.72
8	A-IVh	0.71	0.70
9	A-IVi	0.58	0.59
10	A-IVj	0.58	0.59
11	A-IVk	1.25	1.24
12	A-IVl	0.91	0.91
13	A-IVm	0.71	0.70
14	A-IVn	4.8	4.85
15	A-IVo	2.02	2.1
16	B-IVa	1.25	1.25
17	B-IVb	4.8	4.9
18	B-IVc	1.25	1.29
19	B-IVd	2.02	2.01
20	B-IVe	4.8	4.80
21	B-IVf	4.8	4.79
22	B-IVg	4.8	4.78
23	B-IVh	2.02	2.03
24	B-IVi	2.2	2.21
25	B-IVj	4.8	4.80
26	B-IVk	4.8	4.8
27	B-IVl	1.25	1.27
28	B-IVm	4.8	4.89
29	B-IVn	4.8	4.85
30	B-IVo	2.02	2.01
31	C-IVa	4.8	4.82
32	C-IVb	2.02	2.023
33	C-IVc	4.8	4.87
34	C-IVd	2.02	2.024
35	C-IVe	1.25	1.256
36	C-IVf	2.02	2.021
37	C-IVg	0.91	0.912
38	C-Vh	0.91	0.88
39	C-IVi	0.92	0.92
40	C-IVj	1.25	1.25

3. RESULT

3.1. Note: S- Sensitive R-Resistant

Strain used: ***M. tuberculosis*** (H37 RV strain): ATCC No-27294.

Here are the ***standard values*** for the Anti-Tb test which was performed.

Pyrazinamide-3.125 µg/ml

Streptomycin-6.25 µg/ml

Ciprofloxacin-3.125 µg/ml

3.2. QSAR Analysis

In the present analysis, 26 molecules were used in the training set and 14 molecules in the test set. The 3D QSAR models were generated keeping field grid points not more than seven per model. Tripos force field and Gasteiger-Marsili charges were utilized to calculate electrostatic and steric fields for evaluation of the predictive ability of generated 3D QSAR models. After various combinations of different descriptors, two QSAR models were selected which are shown in Table **5**. The models were selected on the basis of r^2, q^2, pred r^2, F and P values.

3.3. Interpretation of QSAR Model [33]

The QSAR model was selected as the best model among all the developed models to represent the optimum structural requirements of Imidazole analogs to act as antitubercular compound (Figs. **2** & **3**). Optimization of the steric and electronic properties in the imadzole will be having positive influence on the antitubercular activity. The QSAR model A explains better than the QSAR model B. The contributing descriptors in the QSAR model A are the steric interactions at lattice point S_298, S_481, S_682 and electrostatic interaction at lattice point E_431, E_235. The steric interaction at lattice point S_298 is positively contributing to antitubercular activity, so substitution of bulky R-substituent's on the imidazole ring can give increased antitubercular activity, but steric interactions at the lattice points S_481, S_682 are showing negative contribution towards the biological activity, indicating that substitution of aliphatic groups or groups with less steric strain on the aromatic ring of the imidazole nitrogen will increase biological activity. Electrostatic interactions at the lattice point E_431 are contributing positively hence substitution of electron donating substituent on imidazole ring could increase activity and electrostatic interaction at the E_235 lattice point is contributing negatively, showing that substitution of electron withdrawing groups along with this lattice point will increase the electrostatic interaction. Correlation between the observed biological activity and activity predicted by the 3D QSAR model indicates that each of the selected 3D descriptors has appropriate weightage in the selected 3D QSAR equation, representing the correlation of biological activity with these descriptors (Fig. **2 & 3**).

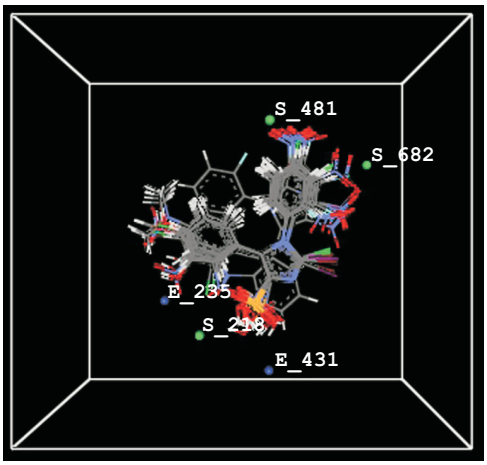


Fig. (2). Field points of selected QSAR model A.

3.4. Pharmacophore Identification Studies [34]

A pharmacophore model is the three-dimensional geometry of interaction features that is necessary for bioactive ligands. A pharmacophore model for Imidazole derivatives as antitubercular agents was generated using the mole sign module of Vlife MDS 4.3. The hypothesis contains characteristic features like aromatic (golden color) and aliphatic regions of the structure (orange color) (Fig. **4**). Imidazole derivatives to act as antitubercular agent should possess two aromatic features 5.015 A ^{0} apart and aliphatic and aromatic feature 4.968 A ^{0} apart.

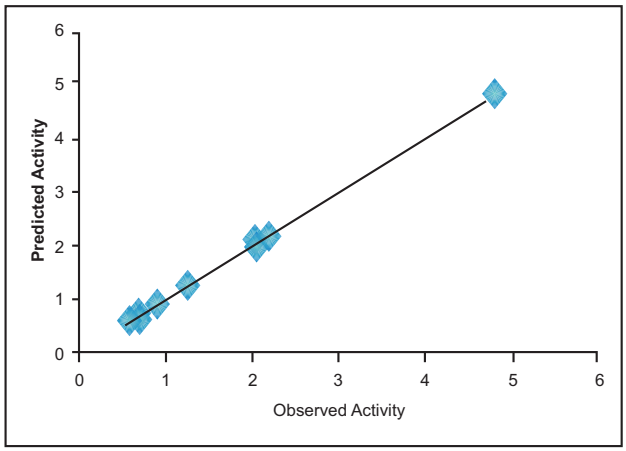


Fig. (3). Correlation graph for selected QSAR model A.

Table 5. QSAR models with statistical information.

Model No.	Model	r^2	q^2	r^2 (Predicted)	F Test
A	pIC_{50} = 1.8408+ 0.2258 E_431-0.1864 E_235+0.0988 S_298-0.8550 S_682-0.0355 S_481	0.95	0.79	0.86	65
B	pIC50 = -7.8024-0.1428 E_592+ 1.0481E_402-0.1250E_613-4.5460S_501-0.0495E_305	0.88	0.81	0.78	56

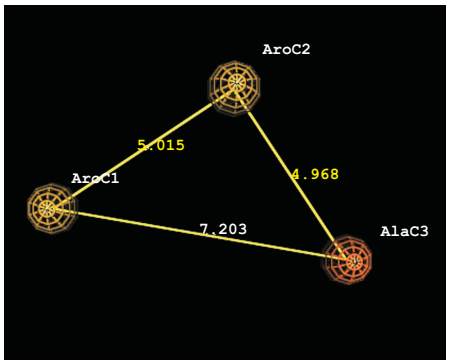


Fig. (4). 3D Projection of Selected Pharmacophore Hypothesis.

3.5. Docking Analysis

Molecular docking analysis was carried out to identify the mode of action of synthesized imidazole derivatives using crystal structure of NADH- dependent enoyl-ACP-reductase (PDBID: 1IDZ) downloaded from rcsb.org. All the synthesized derivatives were docked into the similar binding site. All the binding interactions are summarized in Table **6** as follows (Fig. **5**).

CONCLUSION

The present study showed that the all the synthesized 2,4-disubstituted -1, 5 -diphenyl substituted -1-*H*-imidazole derivatives were evaluated for *in vitro* anti-tubercular activity

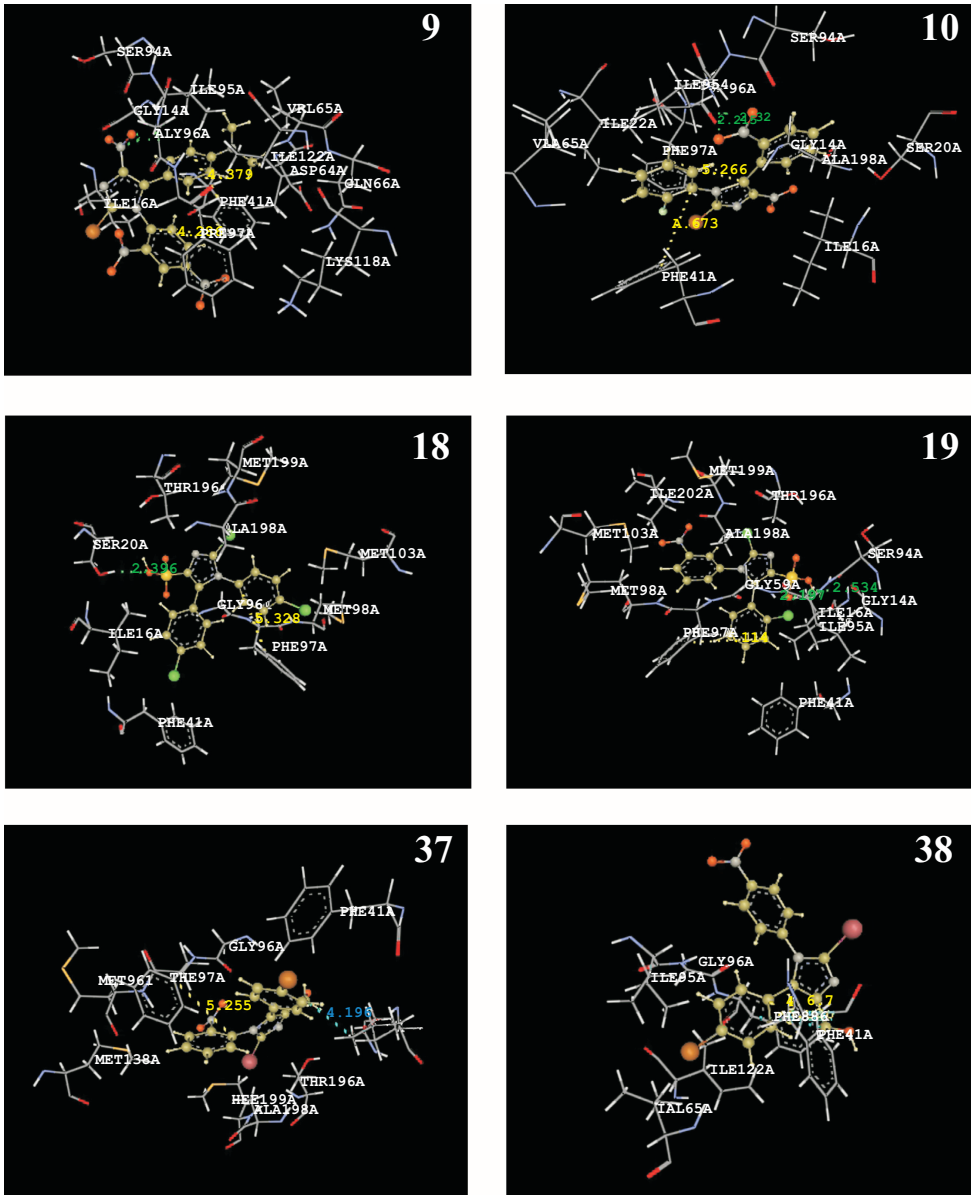


Fig. (5). Docked pose of synthesized molecules in NADH-dependent enoyl- ACP reductase of *Mycobacterium tuberculosis.*

Table 6. Docking study of synthesized molecules.

Sr. No.	Key Molecular Interactions With Amino Acid Involved		
	Hydrogen Bond	**Aromatic**	**Hydrophobic**
1.	PHE97		ARG43, ILE16
2.	GLY96	PHE97	
3.	GLY96	PHE97, PHE41	
4.	GLY96	PHE97, PHE41	ILE122, ILE95, GLN66
5.	VAL65	PHE97, PHE41	
6.	LYS118	PHE97, PHE41	
7.	VAL65	PHE97, PHE41	
8.	VAL65	PHE97, PHE41	
9.	GLY96	PHE97, PHE41	
10.	GLY96	PHE97, PHE41	
11.	ALA198, LEU197, SER20	PHE97	
12.	GLY96, VAL65,	PHE97, PHE41	
13.	GLY96	PHE97, PHE41	
14.	MET98	PHE97	
15.	GLY96	PHE97, PHE41	
16.	GLY96	PHE97, PHE41	
17.	GLY96	PHE97, PHE41	
18.	SER20	PHE97	
19.	GLY96, GLY14	PHE97	
20.	GLY96	PHE97	
21.	SER20		
22.	GLY96	PHE97, PHE41	
23.	ALA22, ILE21	ILE16	
24.	SER94	PHE97	
25.	GLY96	PHE97, PHE41	
26.	MET199	PHE97	ILE16
27.	SER94	PHE97	
28.	GLY96	PHE97, PHE41	ILE122, ILE95, GLN66 VAL65, ASP64, PHE41
29.	GLY96	PHE97, PHE41	
30.	PHE97		
31.	PHE97, PHE41		ILE16
32.	MET98, SER94		SER94, ILE21
33.	SER94		GLY96, ILE95, SER94, GLY14

Table (6) contd….

Sr. No.	Key Molecular Interactions With Amino Acid Involved		
	Hydrogen Bond	**Aromatic**	**Hydrophobic**
34.	MET161	PHE97	MET161, MET103, MET98, PHE97, GLY96
35.	ALA198, LYS118	PHE97, PHE41	ILE16
36.	MET98, ILE15		MET147, SER94, ILE21
37.	PHE97		ILE16
38.		PHE97, PHE41	PHE97
39.		PHE97	THR196
40.	GLY14	PHE97, PHE41	ILE16, GLY14

against *Mycobacterium tuberculosi*s H37Rv strain. Moreover, the improvement in antitubercular activity can be achieved via modification in the substituents on imidazole nucleus. Various recent new drug developments in imidazole derivatives show better effect. QSAR and Pharmacophore analysis also revealed significance of aromatic or groups with increased lipophilicity towards antitubercular activity of imidazole derivatives. This has been observed so far that modification in imidazole moiety displayed important biological activity. It will be exciting to observe that these modifications can be utilized for the development of potent antitubercular derivatives in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Authors would like to thank Dr. Kishore Bhat, Consulting Microbiologist KLEU's, Dr. Prabhakar Kore Basic Science Research Center, for providing facilities to carryout anti-cancer activities, Dr. H. N. More, Principal Bharati Vidyapeeth College of Pharmacy, for providing Computational Chemistry work and Prof. Dr. B. P. Ronge, founder Secretary SVERI Pandharpur, for providing facilities for this research work.

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