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Synthesis, Pharmacological Evaluation and Docking Studies of Newly Synthesized Fluorine Containing 1,2,4-Triazole Clubbed Benzimidazole

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Abstract

The present study involves multistep synthesis sequence to obtain triazole analogues beginning with ethyl tetraflourobenzoate **3** and ethyl pentaflourobenzoate **4**, which on treatment with hydrazine hydrate yields tetra flourobenzoyl hydrazide **5** and penta flourobenzoyl hydrazide **6**. Intermolecular cyclisation of **5**, **6** with substituted heterocyclic acid **7a-h** in presence of phosphorous oxy chloride affords 2-(tetrafluorophenyl)-5-substituted heterocycles-1,3,4-oxadiazole **8a-h** and 2-(pentafluorophenyl)-5-substituted heterocycles-1,3,4-oxadiazole **9a-g**. Condensation of **8a-h** and **9a-g** with 2-hydrazinobenzimdazole **2** results in 3-tetraflourophenyl-5-(substituted heterocycles)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole **10a-h** and 3- pentaflourophenyl-5-(substituted heterocycles)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole **11a-g** analogues. All the synthesized compounds have been established by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data and have been screened for antitubercular activity against $H_{37}Rv$ and antimicrobial activities. Compounds **8d**, **8e**, **8g**, **9b**, **10c**, **10e**, **11b** and **11d** emerged as promising antimicrobials. It was also observed that the promising antimicrobials have proved to be better antituberculars. Compound **10d** showed encouraging antitubercular activity compared to rifampicin. Docking studies of newly synthesized compounds was performed on Crystal structure of Mycobacterium tuberculosis enoyl reductase (INHA) complex with the help of Schrodinger software using Glide to study their observed activity, in which **10d** docked with -9.79 docking score for PDB: 4U0J.

Keywords: 1,2,4-Triazole; Antimicrobial activity; Antitubercular activity; Molecular docking

Introduction

Flourine has played pivotal role in drug discovery. The incorporation of fluorine into a drug modulates the steric and electronic parameters thereby influencing both the pharmacodynamic and pharmacokinetic properties of drugs. In terms of bioisosterism, fluorine acts as an approximate bioisostere of the hydroxyl group. Introducing the carbon–fluorine bond to organic compounds is the major challenge for medicinal chemists using organofluorine chemistry [1], as the carbon–fluorine bond increases the probability of having a successful drug by about a factor of ten. The presence of fluorine often leads to increased lipid solubility, thereby enhancing rates of absorption and transport of drugs in vivo [2]; such as, 5-fluorouracil, fluoxetine (Prozac), paroxetine (Paxil), ciprofloxacin (Cipro), mefloquine, and fluconazole.

The treatment of opportunistic microbial infections has become an important and challenging problem due to the emergence of multiple-drug-resistant organisms [3-6]. Hence, there is an urgent need to develop new classes of agents likely to be unaffected by existing resistance mechanisms. As a part of our ongoing studies to establish new 1,2,4-triazole candidates with improved biological activities [7-10], here in we report the synthesis and pharmacological activities of 3-Tetra/Pentaflourophenyl-5-(substituted heterocycles)-4-(N-1H-benzimidazol-2-amino)-4H-1,2,4-triazole.

The azole antitubercular may be regarded as a new class providing truly effective drugs which are reported to inhibit the bacteria by blocking the biosynthesis of certain bacterial lipids and/or by additional mechanism [11-14]. Triazoles in particular, substituted-1,2,4-triazole are among various heterocycles that have received the most attention during last two decades as potential antimicrobial agents, antifungal, antitubercular, anti-HIV, anti-inflammatory, CNS stimulants, sedatives, antianxiety [15-23]. Benzimidazole moiety has already been reported for its antimicrobial and antitubercular activity along with wide variety of biological activity [24-27]. Prompted by these observations it was contemplated to synthesize a series of 1,2,4-triazole analogues bearing fluorinated moieties to identify new candidates that may bevalue in designing new potent derivatives endowed with various biological activity (Schemes 1 and 2). We also investigated *in silico* binding mode of targeted ligands into the Mycobacterium tuberculosis enoyl reductase (INHA) (**PDB: 4U0J**) complex in comparison with rifampicin as reference drug by docking procedure.

Material and methods

General

All chemical were of analytical grade and used directly. Melting points were determined in PMP-DM scientific melting point apparatus and are uncorrected. The purity of compound was confirmed by TLC using Merck silica gel 60 F_{254} and visualized by exposure to iodine vapours or UV light. IR spectra were recorded on a Perkin-Elmer RX 1 FTIR spectrophotometer, using potassium bromide pellets, the frequencies are expressed in cm^{-1}. The 1H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance II 400 NMR spectrometer, using tetramethylsilane as the internal reference, with chloroform CDCl3 as solvent. The chemical shifts are reported in parts per million (δ ppm). The MS mass spectra were recorded on micromass Q-T of micro (TOF MS ES+).

Biological assay

The MICs of synthesized compounds were carried out by broth microdilution method as described by Rattan [28]. Antibacterial activity was screened against two gram positive bacteria (*S. aureus* MTCC 96, *S. pyogenes* MTCC 443) and two gram negative bacteria (*E. coli* MTCC 442, *P. aeruginosa* MTCC 2488). Ampicillin was used as a standard antibacterial agent. Antifungal activity was screened against three fungal species *C. albicans* MTCC 227, *A. niger* MTCC 282 and *A. clavatus* MTCC 1323. Greseofulvin was used as a standard antifungal agent. The antimicrobial screening data are shown in Table 1 and Table 2.

All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. Inoculum size for test strain was adjusted to 10^8 CFU (Colony Forming Unit) per milliliter by comparing the turbidity. DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains.
MIC of compounds was determined against *M. tuberculosis H37Rv* strain by using Lowenstein-Jensen medium (conventional method) as described by Rattan [28].

In vitro evaluation of antimicrobial activity: The MICs of synthesized compounds were carried out by broth microdilution method. DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37°C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. Each synthesized drug was diluted obtaining 2000 μM concentration, as a stock solution. In primary screening 500, 250 and 125 μM concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, 6.250, 3.125 and 1.5625 μM concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

In vitro evaluation of antitubercular activity: Drug susceptibility and determination of MIC of the test compounds against *M. tuberculosis $H_{37}Rv$* were performed by L.J. agar (MIC) method [29,7-10] where primary 1000, 500, 250 and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25 μM dilutions of each test compound were added liquid L. J medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis $H_{37}Rv$* growing on L. J medium was harvested in 0.85% saline in bijou bottles. All test compound make first stock solution of 2000 μM concentration of compounds was prepared in DMSO. These tubes were then incubated at 37°C for 24 h followed by streaking of *M. tuberculosis $H_{37}Rv$* (5 × 104 bacilli per tube). These tubes were then incubated at 37°C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with M. tuberculosis *$H_{37}Rv$*. The concentration at which no development of colonies occurred or <20 colonies was taken as MIC concentration of test compound. The standard strain M.tuberculosis *$H_{37}Rv$* was tested with known drug Rifampicin and isoniazid.

Molecular docking

On the basis of biological study against *Mycobacterium tuberculosis* strain *$H_{37}RV$*, we selected *Mycobacterium tuberculosis* enoyl reductase (INHA) as a biological target for docking study of newly synthesized compounds. The crystal structure of *Mycobacterium tuberculosis* enoyl reductase (INHA) (PDB: 4U0J) in complex with an irreversible inhibitors was obtained from the RCSB protein data bank [29]. The crystal structure having depth of resolution 1.62 A˚ contains ligand NICOTINAMIDE-ADENINE-DINUCLEOTIDE. The input 3D structures of ligand for all docking experiments were built using the Marvin Suite program and the generated structures were saved as MOL2/SDF files. The energy of ligands was minimised using ligpre programme in maestro Version 10.7.014 (Schrödinger 10.7) software. Proteins were prepared for docking using protein preparation wizard in Glide which includes water removal and energy minimization. The docking simulations were done with the help of ligand docking in glide (Schrodinger 10.7) and docking score, XP GScore, glide evdw, glide ecoul, glide energy, glide emodeland hydrophobic interactions between the protein and the synthesized compounds were recorded. The computational work was performed on a HP Z440 workstation running on Intel xenon processor.

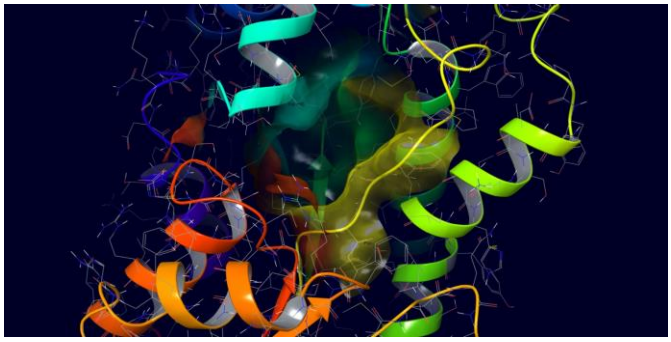


Figure 1: A close-up view of the binding interaction in enoyl-acyl carrier protein reductase (InhA) (PDB ID: 4U0J) with its crystal structure.

Experimental

2-Mercaptobenzimidazole 1 was prepared by the literature procedure [30].

Synthesis of 2-hydrazino benzimidazole 2:

A mixture of 2-mercaptobenzimidazole (25g) and hydrazine hydrate (10 mL) was refluxed for 8-10 h on wire gauze and cooled. The separated crystals were filtered washed with a little amount of ethanol, dried and re-crystallized from ethanol. M.P. 200-201°C.

General process for synthesis of 2-(tetrafluorophenyl)-5-substituted heterocycles-1,3,4-oxadiazole 8a-h and 2-(pentafluorophenyl)-5-substituted heterocycles-1,3,4-oxadiazole 9a-g.

A mixture of tetra flourobenzoylhydrazide 5 (5 mmol) and penta flourobenzoylhydrazide 6 (5 mmol) and various substituted heterocylic acid 7a-h (5 mmol) in phosphorusoxychloride (5 mL) was refluxed on water bath for 6-9 h. The progress of the reaction was monitored by TLC using toluene:ethylacetate:methanol (70:20:10) as mobile phase. After the completion of reaction, it was cooled and poured onto crushed ice with continuous stirring. The solid mass separated was neutralized with sodium bicarbonate solution (10% *w/v*). The resulting solid thus obtained was collected by filtration, washed well with cold water, dried and crystallized from appropriate solvent.

2-Tetraflourophenyl-5-(3-pyridyl)-1,3,4-oxadiazole 8a: Off-white solid (61%); m.p. 278-280°C; IR (KBr): 1658 (C=N), 1276, 1062 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 7.59 (t, 1H, CH), 8.20 (dd, 1H, J = 7.96 Hz, CH), 8.44 (d, 1H, J = 9.56 Hz, CH), 8.76 (dd, 1H, J = 4.4 Hz, CH), 9.29 (s, 1H, CH). ^{13}C NMR (DMSO) δ 165.00 (C_2-oxadiazole), 165.00 (C_5-oxadiazole), 152.23, 148.39,147.26, 142.30, 141.72, 141.01, 134.21, 124.03, 123.38, 122.34, 114.78 (aromatic ring). MS (m/z): 295 (M+).

2- Tetraflourophenyl-5-((2-benzylsulfanyl)3-pyridyl)-1,3,4-oxadiazole 8b: Off-white solid (66%); m.p. 249-251°C; IR (KBr): 1664 (C=N), 1284, 1071 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 4.46 (s, 1H, -CH_2-), 6.89 (t, 1H, CH), 7.26 (t, 1H, CH), 7.33 (t, 2H, CH), 7.40 (t, 2H, CH), 7.86 (d, 1H, CH), 8.19 (dd, 1H, J = 8.04 Hz, CH), 8.27 (d, 1H, CH). ^{13}C NMR (DMSO) δ 164.80 (C_2-oxadiazole), 164.68 (C_5-oxadiazole), 32.81 (-CH_2-), 159.53,147.26, 146.89, 142.30, 141.72, 141.05, 137.18, 132.51, 131.23, 128.78, 127.81, 127.10, 122.34, 119.53, 114.81 (aromatic ring). MS (m/z): 417 (M+).

2- Tetraflourophenyl-5-(furan-2-yl)-1,3,4-oxadiazole 8c: Dull brown solid (65%); m.p. 232-234°C; IR (KBr): 1666 (C=N), 1282, 1070 (C-O-C) cm^{-1}. 1H NMR (DMSO): δ 4.42 (s, 2H, CH_2), 8.20 (dd, 1H, J=7.96 Hz, CH), 8.42 (d, 1H, J=8.35 Hz CH), 8.81 (dd, 1H, J=4.49 Hz, CH). ^{13}C NMR (DMSO) δ 164.58 (C_2-oxadiazole), 157.61 (C_5-oxadiazole), 147.20, 147.32, 142.30, 141.71, 141.05, 138.28, 122.32, 115.53, 114.78, 112.02 (aromatic ring). MS (m/z): 284 (M+).

2-Tetraflourophenyl-5-(7-chloro-1-cyclopropyl-6-fluoro-1*H*-quinolin-4one-3-yl)-1,3,4-oxadiazole 8d: Red solid (67%); m.p. 281-283°C; IR (KBr): 1664 (C=N), 1285, 1072 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 1.08-1.33(m, 4H, cyclopropyl –CH_2-), 4.12 (m, 1H, CH), 6.95 (dd, 1H, J = 4.98 Hz, CH), 7.19 (dd, 1H, J = 8.08 Hz, CH),7.34 (s, 1H, CH) , 8.19 (dd, 1H, J = 8.16 Hz, CH). ^{13}C NMR (DMSO) δ 164.86 (C_2-oxadiazole), 160.34 (C_5-oxadiazole), 7.78 (cyclopropyl-CH_2-), 36.19 (cyclopropyl-CH-), 175.38 (C=O),155.71, 147.23, 142.32, 141.63, 141.02, 127.35, 126.81, 122.30, 118.22, 116.81,114.52, 114.78 (aromatic ring). MS (m/z): 453 (M+), 455 (M+ + 2) .

2-Tetraflourophenyl-5-(1-cyclopropyl-6-fluoro-7-piperazin-1-yl-1*H*-quinolin-4one-3-yl)-1,3,4-oxadiazole 8e: Reddish orange solid (66%); m.p. 238-240°C; IR (KBr): 1656 (C=N), 1279, 1065 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 1.08-1.33(m, 4H, cyclopropyl –CH_2-), 2.01 (s, 1H, piperazine-NH-), 2.78 (t, 4H,- CH_2-), 3.46 (t, 4H,- CH_2-), 4.14 (m, 1H, CH), 6.04 (dd, 1H, J = 4.88 Hz, CH), 7.34 (s, 1H, CH), 8.01 (dd, 1H, J = 7.96 Hz, CH), 8.34 (dd, 1H, J = 8.04 Hz, CH). ^{13}C NMR (DMSO) δ 164.58 (C_2-oxadiazole), 160.31 (C_5-oxadiazole), 7.79 (cyclopropyl-CH_2-), 36.18 (cyclopropyl-CH-), 45.81, 51.35, 175.36 (C=O), 152.51, 147.23, 147.52, 142.35, 141.64, 141.03, 134.52, 122.32, 118.22, 116.81, 114.78, 112.21 (aromatic ring). MS (m/z): 503 (M+).

2-Tetraflourophenyl-5-(1-ethyl-6-fluoro-7-piperazin-1-yl-1*H*-quinolin-4one-3-yl)1,3,4-oxadiazole 8f: Reddish brown solid (69%); m.p. 221-223°C; IR (KBr): 1655 (C=N), 1281, 1068 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 1.31(t, 3H, –CH_3), 1.98 (s, 1H, piperazine-NH-), 2.80 (t, 4H,- CH_2-), 3.48 (t, 4H,- CH_2-), 4.62 (m, 1H, CH), 6.06 (dd, 1H, J = 4.92 Hz, CH), 7.35 (s, 1H, CH), 8.03 (dd, 1H, J = 8.02 Hz, CH), 8.25 (dd, 1H, J = 8.04 Hz, CH). ^{13}C NMR (DMSO) δ 164.60 (C_2-oxadiazole), 160.33 (C_5-oxadiazole), 14.33 (-CH_3), 45.81, 52.12, 51.35, 175.38 (C=O), 152.49, 147.23, 147.54, 142.37, 141.66, 141.04, 139.02, 122.34, 118.24, 116.83, 114.80, 106.31 (aromatic ring). MS (m/z): 491 (M+).

2-Tetraflourophenyl-5-pentaflourophenyl-1,3,4-oxadiazole 8g: Reddis brown (65%); m.p. 284-286°C; IR (KBr): 1659 (C=N), 1279, 1071 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 8.23 (dd, 1H, J = 8.04 Hz, CH). ^{13}C NMR (DMSO) δ 164.53 (C_2-oxadiazole), 164.44 (C_5-oxadiazole), 147.22, 143.94, 142.37, 141.66, 141.04, 138.33, 136.62, 122.33, 114.70, 109.31 (aromatic ring). MS (m/z): 384 (M+).

2,5-(Bistetraflourophenyl)-1,3,4-oxadiazole 8h: Dull brown solid (69%); m.p. 222-224°C; IR (KBr): 1669 (C=N),

1290, 1081 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 8.23 (dd, 2H, J = 7.98 Hz, CH). ^{13}C NMR (DMSO) δ 164.62 (C_2-oxadiazole), 164.55 (C_5-oxadiazole), 147.20, 142.35, 141.64, 141.01, 122.30, 114.73 (aromatic ring). MS (m/z): 366 (M+).

2-**Pentaflourophenyl-5-(3-pyridyl)-1,3,4-oxadiazole 9a:** Dull grey solid (63%); m.p. 267-269°C; IR (KBr): 1659 (C=N), 1278, 1065 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 7.59 (t, 1H, CH), 8.44 (d, 1H, J = 9.56 Hz, CH), 9.29 (s, 1H, CH), 8.76 (dd, 1H, J=4.4 Hz, CH). ^{13}C NMR (DMSO) δ 165.00 (C_2-oxadiazole), 165.00 (C_5-oxadiazole), 152.73, 147.93,143.96, 138.30, 136.62, 134.21, 124.43, 124.02, 109.36 (aromatic ring). MS (m/z): 313 (M+).

2-Pentaflourophenyl-5-((2-benzylsulfanyl)3-pyridyl)-1,3,4-oxadiazole 9b: Off-white solid (64%); m.p. 285-287°C; IR (KBr): 1660 (C=N), 1284, 1069 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 4.46 (s, 1H, -CH_2-), 7.04 (t, 1H, CH), 7.28 (t, 1H, CH), 7.35 (t, 2H, CH), 7.42 (t, 2H, CH), 7.90 (d, 1H, CH), 8.29 (d, 1H, CH). ^{13}C NMR (DMSO) δ 164.58 (C_2-oxadiazole), 164.63 (C_5-oxadiazole), 32.84 (-CH_2-), 159.52, 146.89, 143.98, 138.32, 136.63, 137.16, 132.53, 131.21, 128.76, 127.83, 127.09, 119.52, 109.33 (aromatic ring). MS (m/z): 435 (M+).

2-Pentaflourophenyl-5-(furan-2-yl)-1,3,4-oxadiazole 9c: Greyish white solid (67%); m.p. 220-222°C; IR (KBr): 1669 (C=N), 1288, 1075 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 6.68 (t, 1H, CH), 7.07 (d, 1H, J = 7.36 Hz, CH), 7.86(d, 1H, J = 4.49 Hz, CH),. ^{13}C NMR (DMSO) δ 164.53 (C_2-oxadiazole), 157.65 (C_5-oxadiazole), 147.35, 143.96, 138.33, 138.22, 136.61, 115.51, 112.01, 109.31 (aromatic ring). MS (m/z): 302 (M+).

2-Pentaflourophenyl-5-(7-chloro-1-cyclopropyl-6-fluoro-1*H*-quinolin-4one-3-yl)-1,3,4-oxadiazole 9d: Redish solid (65%); m.p. 238-240°C; IR (KBr): 1661 (C=N), 1273, 1059 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 1.08-1.33(m, 4H, cyclopropyl -CH_2-), 4.13 (m, 1H, CH), 6.97 (dd, 1H, J = 4.94 Hz, CH), 7.20 (dd, 1H, J = 7.98 Hz, CH), 7.35 (s, 1H, CH). ^{13}C NMR (DMSO) δ 164.58 (C_2-oxadiazole), 160.36 (C_5-oxadiazole), 7.78 (cyclopropyl-CH_2-), 36.19 (cyclopropyl-CH-), 175.39 (C=O), 155.73, 143.98, 141.72, 140.92, 138.34, 136.65, 127.34, 126.83, 118.24, 116.82,114.54, 109.33 (aromatic ring). MS (m/z): 471 (M+), 473 (M+ + 2).

2-Pentaflourophenyl-5-(1-cyclopropyl-6-fluoro-7-piperazin-1-yl-1*H*-quinolin-4one-3-yl)-1,3,4-oxadiazole 9e: Reddish brown solid (68%); m.p. 253-255°C; IR (KBr): 1649 (C=N), 1266, 1069 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 1.08-1.33 (m, 4H, cyclopropyl -CH_2-), 1.98 (s, 1H, piperazine-NH-), 2.76 (t, 4H,- CH_2-), 3.45 (t, 4H,- CH_2-), 4.16 (m, 1H, CH), 6.08 (dd, 1H, J = 4.96 Hz, CH), 7.36 (s, 1H, CH), 8.03 (dd, 1H, J = 8.02 Hz, CH). ^{13}C NMR (DMSO) δ 164.58 (C_2-oxadiazole), 160.31 (C_5-oxadiazole), 7.78 (cyclopropyl-CH_2-), 36.20 (cyclopropyl-CH-), 45.83, 51.38, 175.37 (C=O), 152.53, 147.55, 143.98, 141.72, 138.35, 136.65, 134.54, 118.24, 116.83, 112.23, 109.30, 102.51 (aromatic ring). MS (m/z): 521 (M+).

2- Pentaflourophenyl-5-(1-ethyl-6-fluoro-7-piperazin-1-yl-1*H*-quinolin-4one-3-yl)-1,3,4-oxadiazole 9f: Reddish brown solid (63%); m.p. >300°C; IR (KBr): 1669 (C=N), 1282, 1073 (C-O-C) cm^{-1}. ^1H NMR (DMSO): δ 1.33 (t, 3H, –CH_3), 1.99 (s, 1H, piperazine-NH-), 2.82 (t, 4H,- CH_2-), 3.50 (t, 4H,- CH_2-), 4.63 (m, 1H, CH), 6.08 (dd, 1H, J = 4.98 Hz, CH), 7.37 (s, 1H, CH), 8.05 (dd, 1H, J = 8.08 Hz, CH). ^{13}C NMR (DMSO) δ 164.60 (C_2-oxadiazole), 160.33 (C_5-oxadiazole), 14.33 (-CH_3), 45.81, 51.35, 52.12, 175.38 (C=O), 152.49, 147.54, 143.98, 141.72, 138.35, 136.65, 118.24, 116.83, 112.28, 109.33, 106.34 (aromatic ring). MS (m/z): 509 (M+).

2,5-(Bispentaflourophenyl)-1,3,4-oxadiazole 9g: Grey solid (65%); m.p. 285-287°C; IR (KBr): 1667 (C=N), 1287, 1073 (C-O-C) cm^{-1}. ^{13}C NMR (DMSO) δ 164.85 (C_2-oxadiazole), 164.49 (C_5-oxadiazole), 143.98, 138.36, 136.65, 109.36 (aromatic ring). MS (m/z): 401 (M+).

General procedure for the synthesis of 3-tetraflourophenyl-5-(substituted heterocycles)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 10a-h and 3-pentaflourophenyl-5-(substituted heterocycles)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 11a-g.

A mixture of 2-Tetraflourophenyl-5-(3-pyridyl)-1,3,4-oxadiazole 8a (5 mmol) and 2-hydrazinobenzimidazole 2 (5 mmol) in dry pyridine (10 mL) was refluxed for 18-24 h. The reaction was monitored by TLC on silica gel using ethyl acetate:toluene (2.5:7.5). It was then cooled and poured on to crushed ice. The reaction mass was neutralized by dilute hydrochloric acid and resulting solid was washed with cold water, dried and crystallized from ethanol. Yield (57-68 %). The other compounds of the series were prepared by similar procedure.

3-Tetraflourophenyl-5-(3-pyridyl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 10a: Dull grey solid (65%), m.p. 208-210°C; IR (KBr): 3434 (NH), 1649 (C=N) cm^{-1}. ^1H NMR (DMSO) δ 6.76 (t, 2H, CH), 6.91 (t, 2H, CH), 7.59 (t, 1H, CH), 8.20 (dd, 1H, J = 7.96 Hz, CH),7.88 (s, 1H, NH), 8.44 (d, 1H, J = 9.56 Hz, CH), 8.76 (dd, 1H, J = 4.4 Hz, CH), 9.29 (s, 1H, CH), 10.97 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 153.31 (C_3-triazole), 152.83 (C_5-triazole), 152.23, 148.39,147.26, 142.30, 141.52, 141.72, 141.01, 141.52, 136.63, 134.21, 124.03, 123.38, 123.04, 122.34, 115.28, 114.78 (aromatic ring). MS (m/z): 425 (M+).

3-Tetraflourophenyl-5-((2-benzylsulfanyl)-3-pyridyl))-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 10b: Pinkish white solid (61%), m.p. 254-256°C; IR (KBr): 3448 (NH), 1662 (C=N) cm^{-1}. ^1H NMR (DMSO) δ 4.46 (s, 1H, -CH_2-), 6.69 (t, 2H, CH), 6.72 (t, 2H, CH), 6.89 (t, 1H, CH), 7.26 (t, 1H, CH), 7.33 (t, 2H, CH), 7.40 (t, 2H, CH), 7.81 (d, 1H, CH), 7.91 (s, 1H, NH), 8.16 (dd, 1H, J = 7.98 Hz, CH), 10.86 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.48 (C_3-triazole), 151.33 (C_5-triazole), 32.81 (-CH_2-), 159.52,147.27, 146.85, 142.31, 141.70, 141.52, 141.05, 136.63, 137.15, 132.53, 131.21, 128.75, 127.81, 127.15, 122.32, 123.08, 119.57, 115.29, 114.83 (aromatic ring). MS (m/z): 547 (M+).

3-Tetraflourophenyl-5-(furan-2-yl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 10c: Grey solid (62%), m.p. 199-201°C; IR (KBr): 3442 (NH),1660 (C=N) cm^{-1}. ^1H NMR (DMSO) δ 4.46 (s, 2H, CH_2), 8.83 (dd, 1H, J = 5.02 Hz, CH), 8.43 (d, 1H, J = 8.08 Hz, CH), 8.19 (dd, 1H, J =

7.98 Hz, CH), 6.86 (t, 2H, CH), 6.74 (t, 2H, CH), 7.84 (s, 1H, NH), 10.98 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 163.31 (C_3-triazole), 162.83 (C_5-triazole), 147.24, 147.33, 142.32, 141.72, 141.59, 141.08, 138.31, 136.69, 123.08, 122.28, 115.50, 115.21, 114.81, 112.05 (aromatic ring). MS (m/z): 414 (M+).

3-Tetraflourophenyl-5-(7-chloro-1-cyclopropyl-6-fluoro-1*H*-quinolin-4one-3-yl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 10d: Reddish solid (63%), m.p. >300°C; IR (KBr): 3436 (NH),1658 (C=N) cm^{-1}. ^{1}H NMR (DMSO) δ 1.07-1.34 (m, 4H, cyclopropyl –CH_2-), 4.13 (m, 1H, CH), 6.76 (t, 2H, CH), 6.79 (t, 2H, CH), 6.98 (dd, 1H, J = 4.98 Hz, CH), 7.19 (dd, 1H, J = 8.08 Hz, CH), 7.34 (s, 1H, CH), 7.82 (s, 1H, NH), 8.08 (dd, 1H, J = 8.08 Hz, CH), 10.97 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.31 (C_3-triazole), 143.53 (C_5-triazole), 7.78 (cyclopropyl-CH_2-), 36.20 (cyclopropyl-CH-), 175.38 (C=O), 155.73, 147.25, 142.36, 141.50, 141.62, 141.05, 136.68, 127.37, 126.83, 123.08, 122.33, 118.24, 116.83, 115.29, 114.54, 114.80 (aromatic ring). MS (m/z): 583 (M+), 585 (M+ + 2).

3-Tetraflourophenyl-5-(1-cyclopropyl-6-fluoro-7-piperazin-1-yl-1*H*-quinolin-4one-3-yl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 10e: Pink solid (59%), m.p. 286-288°C; IR (KBr): 3441 (NH), 1661 (C=N) cm^{-1}. ^{1}H NMR (DMSO) δ 1.08-1.33(m, 4H, cyclopropyl -CH_2-), 2.01 (s, 1H, piperazine-NH-), 2.78 (t, 4H,- CH_2-), 3.46 (t, 4H,-CH_2-), 4.15 (m, 1H, CH), 6.05 (dd, 1H, J = 5.02 Hz, CH), 6.76 (t, 2H, CH), 6.91 (t, 2H, CH), 7.36 (s, 1H, CH), 7.85 (s, 1H, NH), 8.02 (dd, 1H, J = 8.04 Hz, CH), 8.34 (dd, 1H, J = 8.04 Hz, CH), 10.93 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.59 (C_3-triazole), 143.69 (C_5-triazole), 7.79 (cyclopropyl-CH_2-), 36.18 (cyclopropyl-CH-), 45.81, 51.35, 175.37 (C=O), 152.53, 147.24, 147.55, 141.52, 142.37, 141.66, 141.05, 136.64, 134.56, 123.09, 122.36, 118.25, 116.83, 115.26, 114.81, 112.24 (aromatic ring). MS (m/z): 633 (M+).

3-Tetraflourophenyl-5-(1-ethyl-6-fluoro-7-piperazin-1-yl-1*H*-quinolin-4one-3-yl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 10f: Reddish brown solid (63%), m.p. 268-270°C; IR (KBr): 3449 (NH),1659 (C=N) cm^{-1}. ^{1}H NMR (DMSO) δ 1.31(t, 3H, –CH_3), 1.98 (s, 1H, piperazine-NH-), 2.80 (t, 4H,- CH_2-), 3.48 (t, 4H,- CH_2-), 4.62 (m, 1H, CH), 6.07 (dd, 1H, J = 8.02 Hz, CH), 6.76 (t, 2H, CH), 6.91 (t, 2H, CH), 7.37 (s, 1H, CH), 7.87 (s, 1H, NH), 8.03 (dd, 1H, J = 7.98 Hz, CH), 8.25 (dd, 1H, J = 7.96 Hz, CH), 10.98 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.50 (C_3-triazole), 143.52 (C_5-triazole), 14.36 (-CH_3), 45.83, 52.16, 51.37, 175.40 (C=O), 152.51, 147.21, 147.53, 142.34, 141.62, 141.52, 141.01, 139.05, 136.62, 123.05, 122.36, 118.25, 116.86, 115.29, 114.83, 106.34 (aromatic ring). MS (m/z):

3-Tetraflourophenyl-5-pentaflourophenyl-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 10g: Dull maroon solid (65%), m.p. 265-267°C; IR (KBr): 3449 (NH), 1655 (C=N) cm^{-1}. ^{1}H NMR (DMSO) δ 6.78 (t, 2H, CH), 6.94 (t, 2H, CH), 7.88 (s, 1H, NH), 8.23 (dd, 1H, J = 8.04 Hz, CH), 10.89 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.59 (C_3-triazole), 151.51 (C_5-triazole), 147.22, 143.94, 142.37, 141.66, 141.56, 141.04, 138.33, 136.62, 123.08, 122.33, 115.29, 114.70, 109.31 (aromatic ring). MS (m/z): 514 (M+).

3,5-(Bistetraflourophenyl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 10h: Dull maroon solid (60%), m.p. 238-240°C; IR (KBr): 3447 (NH), 1662 (C=N) cm^{-1}. ^{1}H NMR (DMSO) δ δ 6.78 (t, 2H, CH), 6.93 (t, 2H, CH), 7.81 (s, 1H, NH), 8.23 (dd, 2H, J = 7.98 Hz, CH), 10.96 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.68 (C_3-triazole), 151.53 (C_5-triazole), 147.20, 142.35, 141.64, 141.51, 141.01, 136.68, 123.07, 122.30, 115.25, 114.73 (aromatic ring). MS (m/z): 496 (M+).

3-Pentaflourophenyl-5-(3-pyridyl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 11a: Greyish solid (62%), m.p. 165-167°C; IR (KBr): 3435 (NH), 1660 (C=N) cm^{-1}. ^{1}H NMR (DMSO) δ 6.76 (t, 2H, CH), 6.93 (t, 2H, CH), 7.59 (t, 1H, CH), 7.86 (s, 1H, NH), 8.45(d, 1H, J = 8.98 Hz, CH), 8.76 (dd, 1H, J = 4.48 Hz, CH), 9.29 (s, 1H, CH), 10.98 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.23 (C_3-triazole), 151.18 (C_5-triazole), 152.75, 147.93,143.98, 141.60, 138.32, 136.63, 134.24, 124.45, 124.04, 123.26, 115.25, 109.35 (aromatic ring). MS (m/z): 443 (M+).

3- Pentaflourophenyl-5-((2-benzylsulfanyl)3-pyridyl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 11b: Pale pink solid (61%), m.p. 201-203°C; IR (KBr): 3438 (NH), 1661 (C=N) cm^{-1}. ^{1}H NMR (DMSO) δ 4.46 (s, 1H, -CH_2-), 6.76 (t, 2H, CH), 6.91 (t, 2H, CH), 7.04 (t, 1H, CH), 7.28 (t, 1H, CH), 7.35 (t, 2H, CH), 7.42 (t, 2H, CH), 7.81 (s, 1H, NH), 7.90 (d, 1H, CH), 8.29 (d, 1H, CH), 10.89 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 163.31 (C_3-triazole), 162.83 (C_5-triazole), 32.91 (-CH_2-), 159.53, 146.90, 143.99, 141.56, 138.35, 136.66, 137.21, 132.58, 131.25, 128.79, 127.86, 127.13, 123.12, 119.56, 115.19, 109.36 (aromatic ring). MS (m/z): 565 (M+).

3-Pentaflourophenyl-5-(furan-2-yl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 11c: Greyish solid (68%), m.p. 286-288°C; IR (KBr): 3449 (NH),1652 (C=N) cm^{-1}. ^{1}H NMR (DMSO) δ 6.68 (t, 1H, CH), 6.78 (t, 2H, CH), 6.91 (t, 2H, CH), 7.09 (d, 1H, J = 7.98 Hz, CH), 7.65 (d, 1H, J = 5.04 Hz, CH), 7.89 (s, 1H, NH), 10.95 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 163.31 (C_3-triazole), 162.83 (C_5-triazole), 147.37, 143.98, 141.56, 138.35, 138.25, 136.63, 123.16, 115.19, 115.53, 112.03, 109.34 (aromatic ring). MS (m/z): 432 (M+).

3- Pentaflourophenyl-5-(7-chloro-1-cyclopropyl-6-fluoro-1*H*-quinolin-4one-3-yl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 11d: Redish brown solid (66%), m.p. >300°C; IR (KBr): 3442 (NH), 1659 (C=N) cm^{-1}. ^{1}H NMR (DMSO) δ 1.09-1.34(m, 4H, cyclopropyl –CH_2-), 4.15 (m, 1H, CH), 6.78 (t, 2H, CH), 6.93 (t, 2H, CH), 6.98 (dd, 1H, J = 4.98 Hz, CH), 7.21 (dd, 1H, J = 8.02 Hz, CH), 7.36 (s, 1H, CH), 7.85 (s, 1H, NH), 10.97 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.56 (C_3-triazole), 143.49 (C_5-triazole), 7.81 (cyclopropyl-CH_2-), 36.22 (cyclopropyl-CH-), 175.41 (C=O), 155.75, 144.01, 141.75, 141.56, 140.95, 138.37, 136.67, 127.37, 126.85, 123.05, 118.26, 116.85, 115.28, 114.56, 109.38 (aromatic ring). MS (m/z): 601 (M+).

3-Pentaflourophenyl-5-(1-cyclopropyl-6-fluoro-7-piperazin-1-yl-1*H*-quinolin-4one-3-yl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 11e: Reddish

brown solid (64%), m.p. >300°C; IR (KBr): 3449 (NH),1652 (C=N) cm^{-1}. ^1H NMR (DMSO) δ 1.08-1.33 (m, 4H, cyclopropyl -CH_2-), 1.98 (s, 1H, piperazine-NH-), 2.76 (t, 4H,- CH_2-), 3.45 (t, 4H,- CH_2-), 4.16 (m, 1H, CH), 6.08 (dd, 1H, J = 5.02 Hz, CH), 6.76 (t, 2H, CH), 6.91 (t, 2H, CH), 7.36 (s, 1H, CH), 7.82 (s, 1H, NH), 8.03 (dd, 1H, J = 8.04 Hz, CH), 10.94 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.49 (C_3-triazole), 143.59 (C_5-triazole), 7.78 (cyclopropyl-CH_2-), 36.20 (cyclopropyl-CH-), 45.83, 51.38, 175.37 (C=O), 152.53, 147.55, 143.98, 141.72, 141.50, 138.35, 136.65, 134.54, 123.08, 118.24, 116.83, 115.26, 112.23, 109.30, 102.51 (aromatic ring). MS (m/z): 651 (M+).

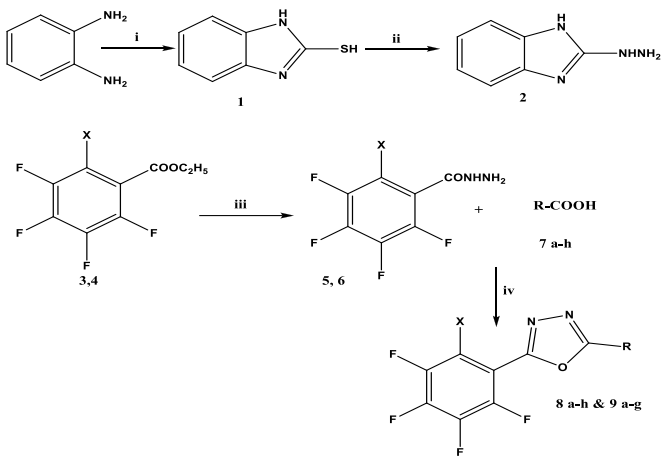
3- Pentaflourophenyl-5-(1-ethyl-6-fluoro-7-piperazin-1-yl-1*H*-quinolin-4one-3-yl)-4-(*N*-1*H*-benzimidazol-2-amino)-4H-1,2,4-triazole 11f: Reddish brown solid (59%), m.p. >300°C; IR (KBr): 3434 (NH),1649 (C=N) cm^{-1}. ^1H NMR (DMSO) δ 1.33 (t, 3H, -CH_3), 1.99 (s, 1H, piperazine-NH-), 2.82 (t, 4H,-CH_2-), 3.50 (t, 4H,-CH_2-), 4.63 (m, 1H, CH), 6.08 (dd, 1H, J = 5.04 Hz, CH), 6.91 (t, 2H, CH), 6.76 (t, 2H, CH), 7.37 (s, 1H, CH), 7.83 (s, 1H, NH), 8.05 (dd, 1H, J = 8.08 Hz, CH), 10.98 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.56 (C_3-triazole), 143.69 (C_5-triazole), 14.33 (-CH_3), 45.81, 51.35, 52.12, 175.38 (C=O), 152.49, 147.54, 143.98, 141.72, 141.53, 138.35, 136.65, 123.03, 118.24, 116.83, 115.29, 112.28, 109.33, 106.34 (aromatic ring). MS (m/z): 639 (M+).

3,5-(Bispentaflourophenyl)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole 11g: Maroon solid (62%), m.p. 188-190°C; IR (KBr): 3432 (NH),1646 (C=N) cm^{-1}. 1H NMR (DMSO) δ 6.76 (t, 2H, CH), 6.91 (t, 2H, CH), 7.89 (s, 1H, NH), 10.92 (s, 1H, benzimidazole NH). ^{13}C NMR (DMSO) δ 151.61 (C_3-triazole), 151.52 (C_5-triazole), 143.98, 141.56, 138.36, 136.65, 123.04, 115.26, 109.36 (aromatic ring). MS (m/z): 532 (M+).

Result and Discussion

Chemistry

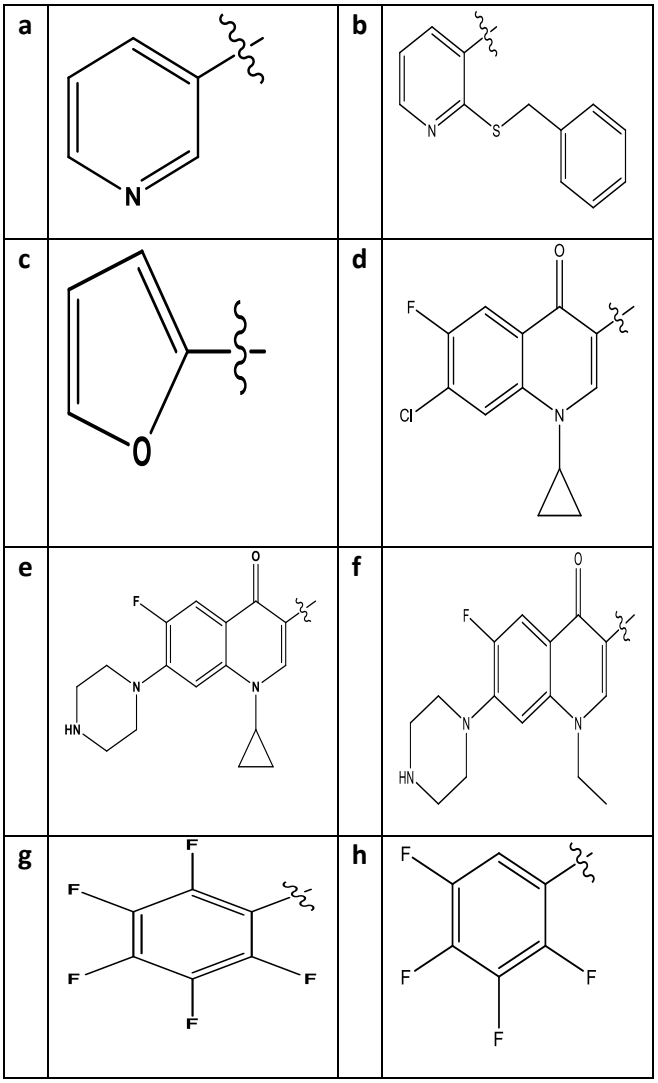
2-Hydrazinobenzimidazole 2 was prepared from substituted 2-mercaptobenzimidazole according to described process as in Scheme 1. Tertaflourobenzoic acid and pentaflourobenzoic acid were converted to ethyl tetraflourobenzoate 4 and ethyl pentaflourobenzoate 5 using ethanol and catalytic amount of sulphuric acid. Ethyl tetraflourobenzoate 4 and ethyl pentaflourobenzoate 5 on treatment with hydrazine hydrate yields tetraflourobenzoyl hydrazide 5 and pentaflourobenzoyl hydrazide 6. Intermolecular cyclisation of tetraflourobenzoyl hydrazide 5 and pentaflourobenzoyl hydrazide 6with substituted heterocyclic acid 7a-h in presence of phosphorous oxy chloride affords 2-(tetrafluorophenyl)-5-substituted heterocycles-1,3,4-oxadiazole 8a-h and 2-(pentafluorophenyl)-5-substituted heterocycles-1,3,4-oxadiazole 9a-g. Condensation of 8a-g and 9a-f with 2-hydrazinobenzimidazole 2 in pyridine results 3-tetraflourophenyl-5-(substituted heterocycles)-4-(N-1H-benzimidazol-2-amino)-4H-1,2,4-triazole 10a-h and 3-pentaflourophenyl-5-(substituted heterocycles)-4-(N-1H-benzimidazol-2-amino)-4H-1,2,4-triazole 11a-g.

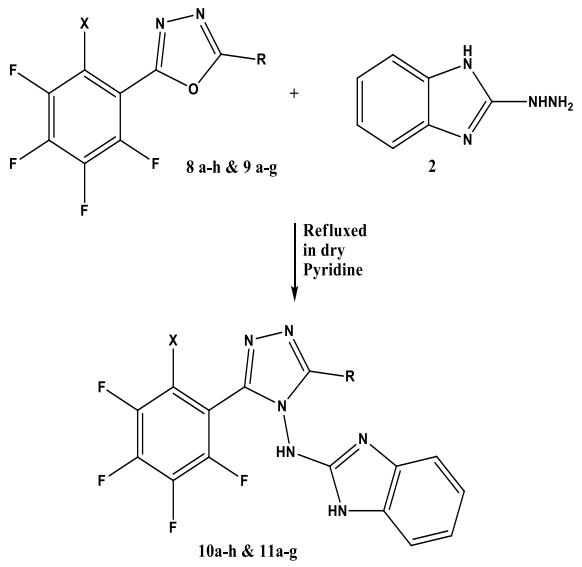


X = H, F i. CS_2, KOH in ethanol, ii. Hydrazine hydrate, iii. Hydrazine hydrate, iv. $POCl_3$

Scheme 1: Synthetic protocol for 2-hydrazinobenzimidazole **2** and 2-Tetra/pentaflourophenyl-5-(substitutedheterocycles)-1,3,4-oxadiazole **8a-h** & **9a-g**.

Scheme-1: Synthesis of intermediate 2, 8a-h and 9a-g.





Scheme 2: Synthetic protocol for 3-Tetra/Pentaflourophenyl-5-(substituted heterocycles)-4-(*N*-1*H*-benzimidazol-2-amino)-4*H*-1,2,4-triazole **10a-h** & **11a-g**.

Scheme 2: Synthesis of final compounds 10a-h and 11a-g.

Analytical results

A series of 1,2,4-triazole analogs have been synthesized in good yields using the synthetic route as outlined in scheme 1. IR, ^{1}H NMR, ^{13}C NMR and mass spectral data are in agreement with the proposed structures of all newly synthesized compounds.

The IR spectra of 1 show the broad stretching band around 3154 and 1519 cm^{-1} where due to amine and thiol. ^{1}H NMR spectrum showed a singlet at δ 6.8 and 12.35 which were accounted for NH and SH.

The IR spectra of 2 showed broad stretching band around 3316 and 3277 cm^{-1} for NH and NH_2. 1H NMR spectrum showed a singlet at δ 4.83 and δ 8.93 which were accounted for NH_2 and NH which vanished on D2O exchange.

In the IR spectrum of tetraflourobenzoyl hydrazide 5 showed stretching band around 3335 and 3278 cm^{-1} where due to amine / amide NH while strong stretching band at 1610 cm^{-1} was attributed to amide carbonyl. ^{1}H NMR spectrum showed a singlet at δ 4.51 and δ 9.81 which were accounted for NH_2 and NH which vanished on D2O exchange.

Lack of 1H NMR resonances observed with NH and NH_2 functions in the 1H NMR spectrum of 5 proved that ring closure starting from 7a, resulted in the formation of 1,3,4-oxadiazole ring. This was further substantiated by the ^{13}C NMR data of 8a which showed a peak at δ 165.00 due to C_2 and C_5 of oxadiazole. Mass spectrum of 8a displayed a molecular ion base peak at m/z 295 which confirmed its molecular weight.

Lack of 1H NMR resonances observed with NH_2 function in the 1H NMR of 10a proved the condensation of 2 and 8a resulted in the formation of 1,2,4-triazole ring. This was substantiated by ^{13}C NMR data of 10a which showed a peak at δ153.31 and δ 152.83 due to C_2 and C_5 of triazole. Mass spectrum of 10a displayed a molecular ion base peak at m/z 425 which confirmed its molecular weight.

Biological results

The antibacterial screening results are shown in Table 1. The results revealed that 1,3,4-moderately active against bacteria except 8g, which showed good activity (50 μM) as compared with ciprofloxacin against *S. aureus* and *S. pyogenus* while compound 8d and 8e showed good activity (25 μM) and compound 9b showed excellent activity (12.5 μM) as compared with ciprofloxacin against *E. coli*. Most of 1,2,4-triazole derivative were found good to moderate active (62.5-125 μM) against *S. aureus*. Compound 10e possessed good activity (50 μM) and 11b possessed excellent activity (12.5 μM) against *S. aureus*.

Compound	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*S. pyogenus*	*E. coli*	*P. aeruginosa*
	MTCC-96	MTCC-443	MTCC-442	MTCC-2488
2	500	125	50	50
8a	500	250	100	250
8b	125	250	125	125
8c	250	250	100	125
8d	125	125	25	125
8e	250	125	25	100
8f	100	125	100	125
8g	50	50	250	125
8h	250	125	125	125
9a	125	250	100	125
9b	250	200	12.5	50
9c	500	135	250	100
9d	125	100	250	125
9e	125	250	50	100
9f	250	125	125	125
9g	250	100	125	125
10a	125	125	125	250
10b	125	125	125	125
10c	100	125	125	125
10d	500	250	250	250
10e	50	250	250	250
10f	125	125	250	250
10g	250	100	125	100
10h	250	100	250	50
11a	100	100	100	250
11b	25	125	62.5	50
11c	250	250	100	125
11d	250	50	100	50
11e	250	250	62.5	125
11f	250	125	125	125
11g	125	125	100	250
Ciprofloxacin	50	50	25	25

Table 1: Antibacterial activity (MICs, μM) of compounds 2, 8a-h, 9a-g, 10a-h and 11a-g.

All the compounds exhibited moderate activity (50-125 μM) except 11d against *S. pyogenus*. All the compounds showed moderate to weak activity against *P. aeruginosa*.

Compound 8g, 10e, 11b and 11d exhibited very good activity against gram positive bacteria whereas 8d, 8e and 9b showed very good activity towards gram negative bacteria. None of the compound was found active against both gram positive and gram negative bacteria.

The results of antifungal activity are summarized in Table 2. The results showed that 1,3,4-oxadiazoles 8c and 1,2,4-triazoles 10b, 10c, 10h, 11e and 11g possessed good activity (250 μM) against C. albicans whereas 8e, 10e, 10d, 11b, 11c, 11d and 11f showed better activity (100 μM) against C. albicans. All compounds displayed moderate to weak activity (250-500 μM) against *A. niger* and *A. clavatus* whereas compounds 9f, 11b (100 μM) and 10c (62.5 μM) exhibited good activity against *A. niger*. 1,3,4-oxadiazole 8g exhibited weak activity against all three fungi. Compound 10c having furyl linkage on 1,2,4-triazole showed better activity.

Compound	Fungal species		
	C. albicans	*A. niger*	*A. clavatus*
	MTCC-227	MTCC-282	MTCC-323
2	500	1000	500
8a	500	1000	250
8b	500	1000	1000
8c	250	1000	1000
8d	500	1000	1000
8e	100	500	250
8f	1000	250	250
8g	1000	1000	1000
8h	500	500	1000
9a	500	500	500
9b	500	500	250
9c	500	500	250
9d	500	500	1000
9e	1000	500	250
9f	1000	100	250
9g	500	500	500
10a	500	500	250
10b	250	1000	1000
10c	250	62.5	250
10d	100	500	500
10e	100	500	500
10f	500	250	250
10g	500	500	500
10h	250	1000	1000
11a	500	250	500
11b	100	100	250
11c	100	500	500
11d	100	500	500
11e	250	250	500
11f	100	1000	1000
11g	250	1000	1000
Greseofuvin	500	100	100

Table 2: Antifungal activity (MICs, μM) of compounds 2, 8a-h, 9a-g, 10a-h and 11a-g.

The encouraging results from the antibacterial studies impelled us to go for preliminary screening of synthesized compounds against M. tuberculosis is summarized in Table 3. Compound 8d, 9b, 9d and 10b showed good activity (25-50 μM) which is attributed due to quinolonic, ciprofloxacin and norfloxacin linkage on oxadiazole whereas compound 10d (12.5 μM). Due to the better activity against tested microorganisms and mycobacteria, compound 10d has been selected for further development and studies to acquire more information about structure activity relationships are in progress in our laboratories.

Synthesized compounds and standard drug (Rifampicin) were docked into the active site of *Mycobacterium tuberculosis* enoyl reductase (INHA) (PDB ID: 4U0J) of *Escherichia coli* [31] using Glide package in maestro Schrodinger 10.7 software to understand the binding interactions.

Compound	MIC values (μM)	% Inhibition
8a	500	97%
8b	250	99%
8c	1000	98%
8d	50	99%
8e	250	99%
8f	125	98%
8g	100	98%
8h	100	99%
9a	100	99%
9b	25	98%
9c	200	98%
9d	50	99%
9e	250	98%
9f	125	99%
9g	100	99%
10a	100	99%
10b	50	98%
10c	100	99%
10d	12.5	99%
10e	250	99%
10f	100	98%
10g	62.5	99%
10h	62.5	98%
11a	100	99%
11b	500	99%
11c	250	99%
11d	100	98%
11e	500	99%
11f	125	99%
11g	100	99%
Econazole	12.5	99%
Isoniazid	0.2	99%
Rifampicin	40	99%

Table 3: Minimal inhibitory concentrations (MICs, □M) against *M .tuberculosis* $H_{37}R_v$ Docking studies.

The data obtained from docking study is presented in Table 4. In molecular docking analysis, hydrogen bonding interactions, hydrophobic interaction, van der Waals interactions and p–p interactions were examined through various targets. Docking of the ligands to their receptors showed a RMSD value less than 2A˚ with binding energy -38.019 kcal/mol. The docking results indicated compounds 10d shows very good binding energy in the active pocket of receptor. Compound 10d form the interactions with amino

acid residues GLY14, ALA22, SER94, PHE149, ALA157 and VLA28. The compounds 9d had shown hydrogen bonding interaction with amino acid residues SER94 and GLY96 respectively. The halogen substituted compounds 10d and 9d were more favorable for hydrophobic interactions as compare to other substituted compounds. The Cl and F substituent at benzene ring of most active compound 10d fitted well into the hydrophobic pocket. On the basis of activity data and docking result, it was found that compound 10d, 9b, 9d, 10b and 8b had potential to inhibit enoyl reductase of *E. coli*. Figure 2a, 3a, 4a and 5a shows the fit of compound 9b, 9d, 10b and 10d into active site of the receptor respectively. The 2D plots of ligand interaction map are shown in Fig. 2b, 3b, 4b and 5b for the compound 9b, 9d, 10b and 10d respectively.

Compound	Docking score	XP GScore	Glide evdw	Glide ecoul	Glide energy	Glide emodel
PDB ID-4U0J						
8a	-8.011	-8.013	-28.321	-2.759	-31.08	-44.775
8b	-9.097	-9.098	-35.935	-1.149	-37.085	-58.785
8c	-8.056	-8.056	-25.396	-1.213	-26.609	-35.015
8d	-8.454	-8.454	-38.6	-1.678	-40.277	-63.548
8e	-7.235	-7.729	-40.48	-0.114	-40.594	-63.307
8f	-8.044	-8.049	-36.262	0.907	-35.356	-55.49
8g	-8.399	-8.399	-27.613	-1.742	-29.356	-47.267
8h	-8.316	-8.316	-23.43	-1.802	-25.232	-45.122
9a	-8.288	-8.29	-26.966	-3.139	-30.105	-41.507
9b	**-9.488**	-9.489	-40.747	-2.64	-43.386	-64.152
9c	-8.09	-8.09	-25.536	-1.43	-26.966	-36.848
9d	**-9.362**	-9.362	-39.342	-0.93	-40.272	-61.612
9e	-4.462	-4.467	-41.326	-0.347	-41.674	-65.007
9f	-3.798	-3.804	-41.566	-0.96	-42.526	-64.868
9g	-7.51	-7.51	-27.886	-1.867	-29.754	-45.277
10a	-7.849	-7.855	-34.02	-0.309	-34.329	-58.306
10b	**-9.237**	-9.237	-49.894	2.114	-47.78	-80.136
10c	-7.893	-7.896	-31.25	-2.534	-33.784	-57.39
10d	**-9.79**	-9.792	-47.021	-1.47	-48.491	-79.373
10e	-6.547	-6.552	-51.291	2.068	-49.224	-75.875
10f	-5.508	-5.514	-43.116	0.326	-42.79	-73.141
10g	-8.841	-8.844	-33.814	-2.509	-36.323	-63.367
10h	-8.44	-8.442	-37.367	-2.122	-39.489	-63.883
11a	-8.039	-8.044	-34.186	0.081	-34.104	-57.655
11b	-7.723	-7.723	-53.023	-4.749	-57.772	-86.271
11c	-7.223	-7.226	-31.816	-0.914	-32.73	-55.239
11d	-5.991	-5.993	-51.152	1.39	-49.762	-76.173
11e	-4.874	-4.88	-45.468	-5.897	-51.365	-76.67
11f	-4.519	-4.525	-48.33	2.703	-45.627	-73.932
11g	-8.44	-8.442	-37.367	-2.122	-39.489	-63.936
Econazol	-7.302	-8.025	-8.025	-34.596	-4.423	-39.019
Isoniazid	-6.031	-6.032	-6.032	-19.516	-2.779	-22.295
Rifampicin	-2.38	-4.299	-4.299	-43.412	-4.209	-47.62

Table 4: Results of the molecular docking study; Glide score & Glide energy (kcal/mol) of the compounds 8a-h, 9a-g, 10a-h and 11a-g.

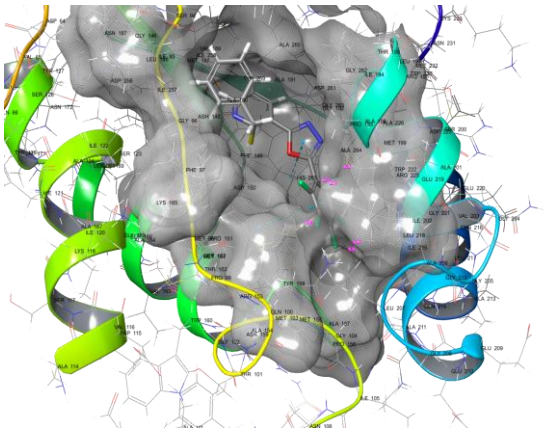


Figure 2a: 3D presentation of hydrogen bond interactions of a compound 9b into the active site of *Mycobacterium tuberculosis* enoyl reductase (INHA) (PDB ID: 4U0J).

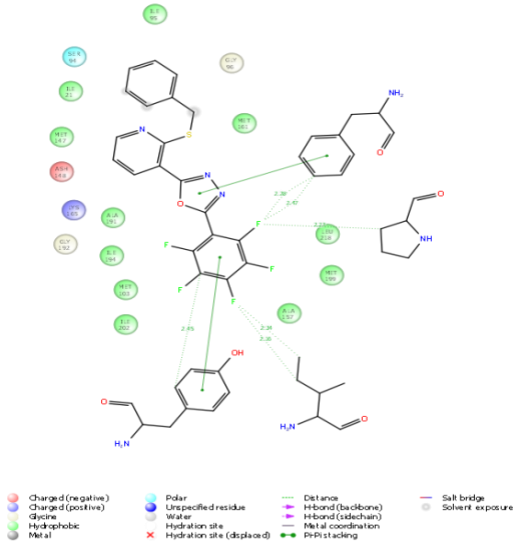


Figure 2b: 2D presentation of ligand 9b interacting with amino acid residues.

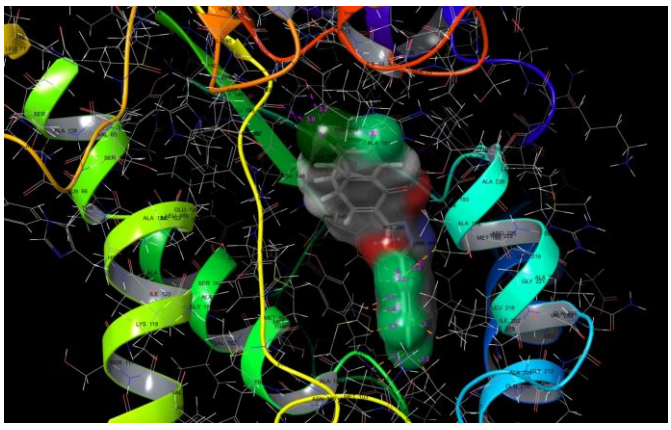


Figure 3a: 3D presentation of hydrogen bond interactions of a compound 9d into the active site of *Mycobacterium tuberculosis* enoyl reductase (INHA) (PDB ID: 4U0J).

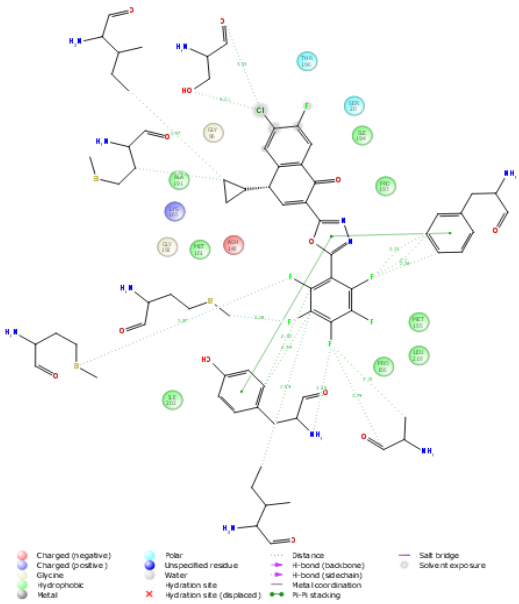


Figure 3b: 2D presentation of ligand 9d interacting with amino acid residues.

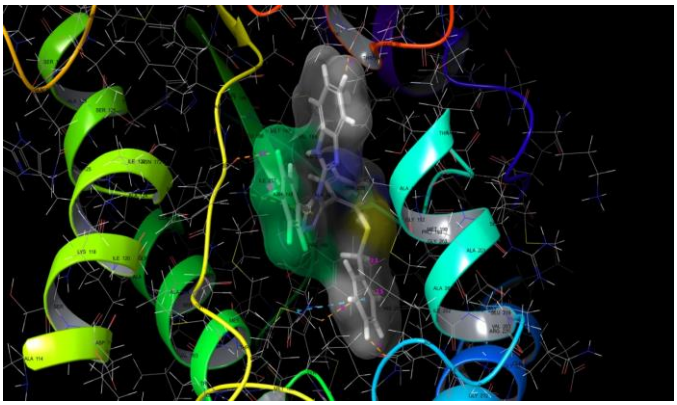


Figure 4a: 3D presentation of hydrogen bond interactions of a compound 10b into the active site of *Mycobacterium tuberculosis* enoyl reductase (INHA) (PDB ID: 4U0J).

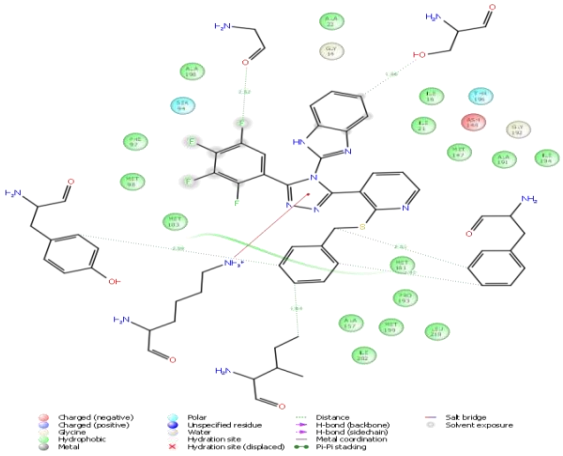


Figure 4b: 2D presentation of ligand 10b interacting with amino acid residues.

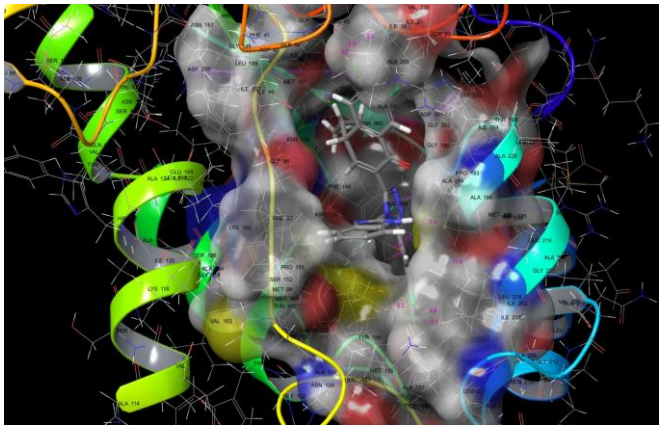


Figure 5a: 3D presentation of hydrogen bond interactions of a compound 10d into the active site of *Mycobacterium tuberculosis* enoyl reductase (INHA) (PDB ID: 4U0J).

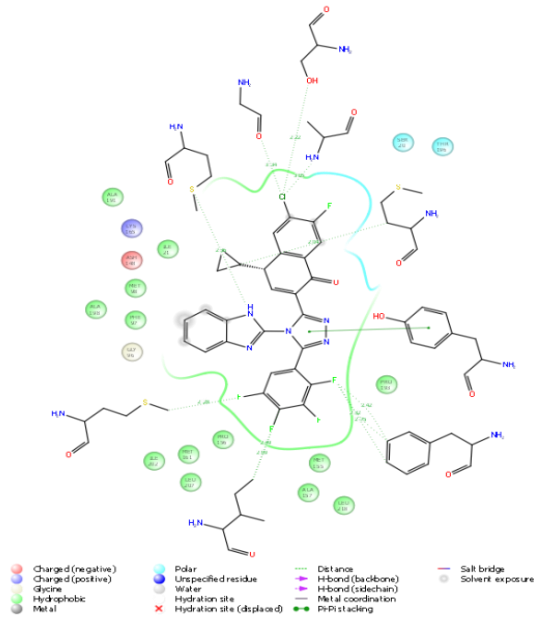


Figure 5b: 2D presentation of ligand 10d interacting with amino acid residues.

Conclusion

A series of newer analogs of 1,2,4-triazoles were synthesized by introduction of 2-hydrazinobenimidazole to 1,3,4-oxadiazole and assessed for their antimicrobial and antituberculosis activity. Modification of heterocyclic linkage on C-5 position of 1,3,4-oxadiazole with various biologically active ring system improved the activity. The antibacterial data indicates that the analogs with (2-benzylsulfanyl)3-pyridyl, quinoloyl and ciprofloxacin linkage as promising antimicrobials showing better to moderate activity while analogs bearing furyl linkge showed better antifungal activity. It was also observed that the promising antimicrobials have proved to be better antituberculars. Specially, compound 10d due to their better activity against *H37Rv* strain, are the best choice for the preparation of new derivatives in order to improve antitubercular activity in future.

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