INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

NOVEL 2,3-DISUBSTITUTED BENZOFURAN DERIVATIVES AS POTENTIAL INHIBITORS OF CHORISMATE MUTASE ENZYME

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ABSTRACT

Novel 3-amino-benzofuran-2-hydrazide ligand was identified for *Mycobacterium tuberculosis* Chorismate Mutase (*MtbCM or CM) inhibition and synthesized few of its analogues and characterised spectroscopically. All the isolated compounds were screened *in-vitro* inhibition activity against CM, have showed potential inhibition when tested with 30 µM. Based on preliminary investigation on CM, compound **7d** was revealed most potential inhibition 84% at 30 µM. Also exhibited growth inhibition on gram+ve bacteria (MIC values are in 3.9 to 15.6 µg/mL). So 3-amino-benzofuran-2-hydrazone derivatives are the most promising lead compound towards further development of its new series for potential inhibitors of CM and antimicrobial agents.

Keywords: Benzofuran, Urea, Amide, Sulphonamide, Hydrazide, Hydrazone, Chorismate Mutase (CM).

1. INTRODUCTION

Tuberculosis (TB) the major threat to human health globally, due to limited available drugs with long term regimen. TB is one of the foremost infectious diseases caused by *Mycobacterium tuberculosis* strain (Mtb). Some of the countries due to low poverty and lack of hygienic life style the disease is being spread quickly. There were estimated 10 million of new TB cases worldwide and 10 to 15% fatalities. Where in most of the mortalities are being recorded due to its co-morbidity HIV-AIDS, dropped efforts in anti-infective research and mainly re-occurrence as drug resistance (multi or extensive drug resistance MDR or EDR)TB to first line medicines due to irregular medication or incomplete regimen due to in-affordable. Over the last few decades the research on TB is being initiated by pharmaceutical companies and charities is encouraged us to be part of the development of new series of anti-tubercular drugs to control the number of TB incidents by minimising the toxicity, regimen and the cost of treatment. Current research on molecular biology was keen to establish the *MtbCM (PDB code 2FP2) was isolated from microorganisms. Indeed the protein-ligand binding energies via *in-silico* models are being very useful to find a new series of pharmacophore to inhibit CM. This will catalyse the conversion of Chorismate into prephenate in Shikimate biosynthetic pathway to produce tyrosine and phenylalanine. These amino acids are essential to survive *Mycobacterium tuberculosis* therefore inhibition of CM may stop producing these two amino acids in the microorganism which leads to death of microorganism. Since CM, shikimate biosynthetic pathway (Fig-1) is absent in humans but not in microorganisms was reflected as research target for the recognition of new drugs for infectious diseases. In current years there has been an improved attention on substituted benzofuran as antibacterial, anti-fungal, and anti-cancer agents[1]. Benzofuran class of fused heterocycles has been of great interest to

explore a new class of broad spectrum antibiotics against pathogens[2-4]. It has been demonstrated that aryl hydrazones are an important and promising class of anti-infective agents[5]. 3-Arylaminobenzofuran[6] derivatives were exhibited antiproliferative activity against cancer cells in culture at nanomolar concentrations (IC_{50} values of 0.3–27 nM) and also found to have inhibition of tubulin polymerization.

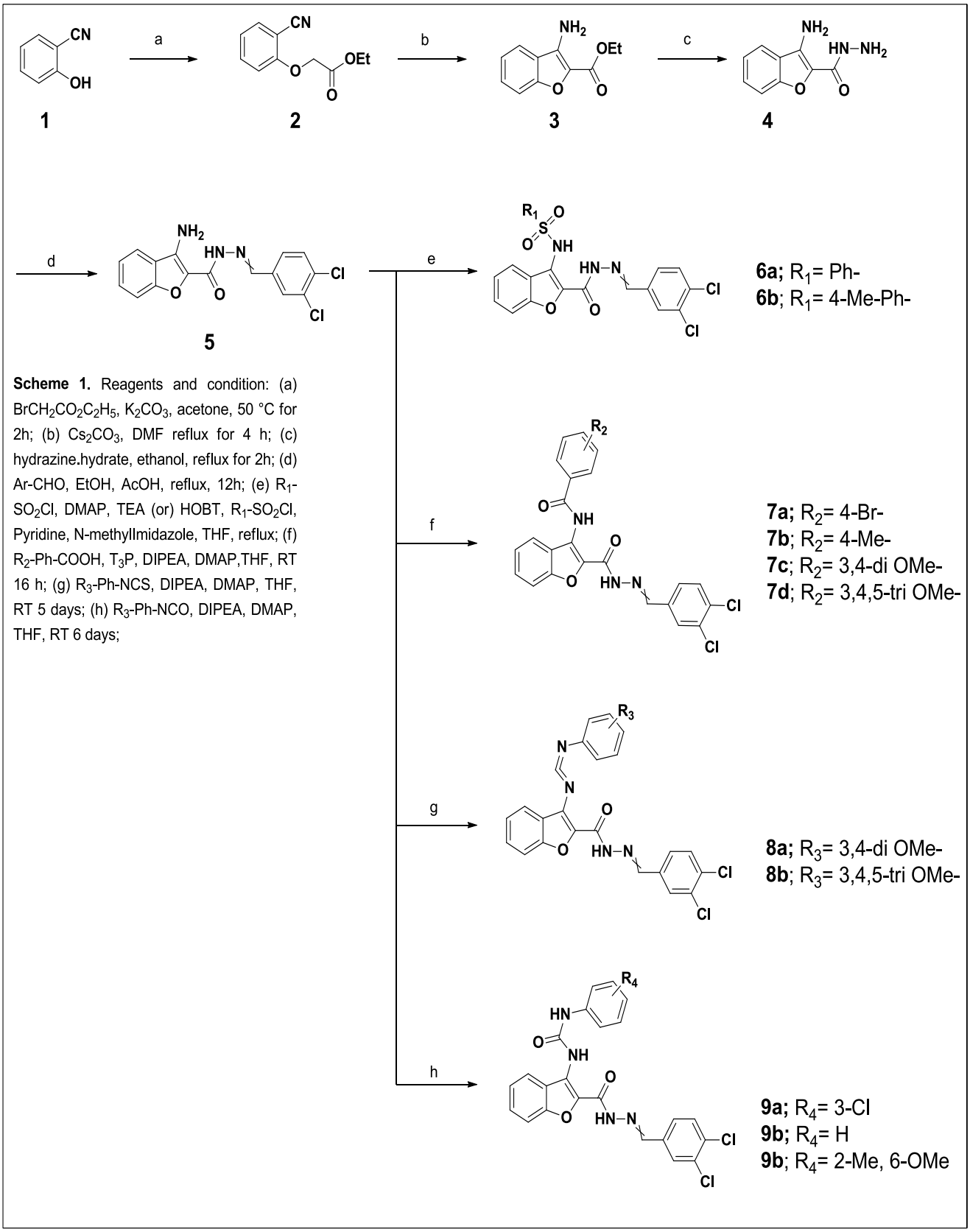
In recent years the new concept Molecular hybridization in drug design is found to be most efficient to identify the lead compounds for better efficacy and affinity. Two different active fragments combines together to produce a new pharmacophore for better receptor binding through virtual screening followed by *in-vitro* high throughput experimental screening to get a lead compound was encouraged us towards design of 2,3-disubstituted benzofuran ligand for anti-tuberculosis and antimicrobial agents. DHPM framework[7] was found to be most potent inhibitory effect on CM which will hinder the supply of essential amino acids to the organism.

Isoniazid and Rifampicin (Fig-2) are foremost prescribed drugs for treating tuberculosis and their fragments hydrazide from isoniazid and benzofuran from Rifampicin are most interesting fragments to combine to get a novel 3-amino benzofuran-2-hydrazide scaffold to extend our research further to evaluate *in-vitro* CM inhibition. However, only a few small molecules have been reported to possess inhibitory activity against CM[8-10]. In continuation of our efforts on the identification of novel inhibitors of CM, we became interested in evaluating the library of small molecules based on 3-amino-benzofuran-2-acylhydrazone framework.We herein report the design, synthesis and biological evaluation of novel benzofuran derivatives as a new hybrid molecule with sulphonamides, benzamides and urea's as its derivatives to identify more potent biologically active compounds. Compound **6b** was found to have better glide score (deltaGligsolvpol: -16.40 using SwissDock software) which have been showed strong hydrogen bonding interactions involved the secondary amide substitution on 03rd position of benzofuran and hydrazone at 02nd position with Arg49, Lys60, Glu109 and Gln76 residues of the Chorismate Mutase protein (Fig-3). Remarkably shifting of amine and hydrazones groups on to the other positions of benzofuran or removal of either group have decreased the interactions with the CM protein *in silico*. These annotations were strengthening our initial thought to focus on 3-amino-benzofuran-2-hydrazone containing benzene sulphonamide, benzamide and phenyl urea's on 3-amino group and 3,4-dichloro phenyl hydrazone at 02nd position are essential to retain the interactions with specified amino acids in the CM protein model. As best of our knowledge 3-amino-N'-(3,4-dichlorobenzylidene) benzofuran-2-carbohydrazide **5** derivatives are most potential inhibitors of CM.

2. MATERIALS AND METHODS

Experimental

In the present work, proposed sulphonamides, benzamides and urea hybrids were synthesized utilizing the reaction sequence as shown in Scheme 1. Alkylation of 2-hydroxy benzonitrile using equimolar quantity of ethyl bromoacetate in presence of K_2CO_3/Cs_2CO_3 in dry acetone/dimethylformamide gave an excellent yield of ethyl 3-amino benzofuran-2-carboxylate **3**. Ethyl 3-amino-benzofuran-2-carboxylate **3** on stirring with excess hydrazine hydrate in ethanol at reflux temperature gave 3-amino-benzofuran-2-carbohydrazide[11] **4** in good yield. Compound **4** on condensation with 3,4-dichloro-benzaldehyde in presence of catalytic amount of glacial acetic acid (or) activated 4Å molecular sieves to give hydrazone compound **5**. Regioselectivity of compound **5** was confirmed by ^{1}HNMR, HMBC, HSQC and NOESY experiments. This was used further to generate sulphonamides (**6a**-**6b**) with sulfonyl chlorides, benzamides (**7a-7d**) with carboxylic acids, ketenes (**8a** & **8b**) with thioisocyanates and urea's (**9a-9c**) with isocyanates in presence of DiPEA, DMAP in THF at room temperature for 6 days. All the final compounds were purified by reverse phase Preparative-HPLC (using C-18 column, 0.5% HCOOH in water and acetonitrile) the fractions were lyophilised to give free flowing pale yellow colour solids.



Scheme 1. Reagents and condition: (a) $BrCH_2CO_2C_2H_5$, K_2CO_3, acetone, 50 °C for 2h; (b) Cs_2CO_3, DMF reflux for 4 h; (c) hydrazine.hydrate, ethanol, reflux for 2h; (d) Ar-CHO, EtOH, AcOH, reflux, 12h; (e) R_1-SO_2Cl, DMAP, TEA (or) HOBT, R_1-SO_2Cl, Pyridine, N-methylImidazole, THF, reflux; (f) R_2-Ph-COOH, T_3P, DIPEA, DMAP,THF, RT 16 h; (g) R_3-Ph-NCS, DIPEA, DMAP, THF, RT 5 days; (h) R_3-Ph-NCO, DIPEA, DMAP, THF, RT 6 days;

Commercially available chemicals and solvents were purchased from Sigma Aldrich and were used without further purification. All the reactions were conducted in oven dried glassware under an argon atmosphere, THF was dried using Na metal and benzophenone, DMF was used as dry solvent and stored on molecular sieves (4Å). TEA was stored over KOH. All the reactions progress was monitored by thin layer chromatography (TLC)

on silica gel plates (60 F_{254}), visualizing with UV-light (254 nm) or iodine spray and then flash column chromatography was carried out with Merck silica gel 100-200 mesh as stationary phase. NMR spectra were recorded on a Bruker400 spectrometer (or otherwise stated) at 400 MHz for ^{1}H and 100 MHz for ^{13}CNMR in DMSO-d_6, $CDCl_3$, & CD_3OD. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), dt (doublet of triplet), br s (broad singlet), br d (broad doublet) and coupling constants are reported in Hertz (Hz). Chemical shifts (d) are reported in ppm relative to the residual solvent peak. Elucidations of chemical structures were based on ^{1}H NMR, ^{13}CNMR, NOESY experiments. Mass spectra were recorded on a Micromass LC-TOF instrument by using electrospray ionization (ESI). HRMS was determined using waters LCT premier XETOF ARE-047 apparatus. Concentrations are reported in gram per 100 mL. Melting points were measured on a BüchieM 565 and IR values were recorded on ShimadzueIR affinity-1 using KBr pellet. The cell culture chemicals used to determine the CM inhibition effect were purchased from Gibco.

General procedure for the synthesis of 2-(2-cyanophenoxy)acetate (2)

To a stirred suspension of 2-Hydroxy benzonitrile **1** (20 g, 167.89 mmol), K_2CO_3 (69.61 g, 503.69 mmol) in dry acetone (250 mL) under argon atmosphere was added ethyl bromoacetate (33.64 g, 201.46 mmol) dropwise. After stirring for 2 h at 50 °C, the reaction mixture was evaporated to dryness. The crude compound obtained was partitioned between ethyl acetate and cold water. The combined organic extracts were washed with brine solution and dried over anhydrous sodium sulphate. After filtration and removal of the volatiles under reduced pressure, the residue obtained was purified by flash column chromatography using petroleum ether/ethyl acetate (10:1) as an eluent to afford compound **2** (29.64 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J*=1.5, 7.8 Hz, 1H), 7.54 - 7.49 (m, 1H), 7.06 (t, *J*=7.6 Hz, 1H), 6.84 (d, *J*=8.3 Hz, 1H), 4.76 (s, 2H), 4.26 (q, *J*=6.8 Hz, 2H), 1.28 (t, *J*=7.1 Hz, 3H); FT-IR: 2238 (CN), 1721 (C=O) cm^{-1}.

General procedure for the synthesis ofethyl 3-aminobenzofuran-2-carboxylate (3)

To a stirred suspension of ethyl 2-(2-cyanophenoxy)acetate **2** (29 g, 141.31 mmol), K_2CO_3 (39.06 g, 282.63 mmol) in dry N,N-dimethylformamide (300 mL) under argon atmosphere was heated at 140 °C for 4 h. The reaction mixture was evaporated to dryness. The crude compound obtained was partitioned between ethyl acetate and cold water. All the combined organic extracts were washed with brine solution and dried over anhydrous Na_2SO_4. After filtration and removal of the volatiles under reduced pressure, the residue obtained was purified by flash column chromatography (silica-gel, 100-200 mesh) using petroleum ether/ethyl acetate (7:3) as an eluent to afford **3** (21.54 g, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J*=7.8 Hz, 1H), 7.46 (d, *J*=3.9 Hz, 2H), 7.26 (m, 1H), 4.98 (b s, 2H), 4.45 (q, *J*=6.8 Hz, 2H), 1.44 (t, *J*=7.1 Hz, 3H); FT-IR: 1712 (C=O) cm^{-1}; MS m/z 206.23 $(M+H)^{+}$.

General procedure for the synthesis of 3-aminobenzofuran-2-carbohydrazide (4)

To a stirred suspension of ethyl 3-aminobenzofuran-2-carboxylate **3** (21 g, 102.33 mmol), hydrazine hydrate (80% aq. solution, 25.58 mL, 409.32 mmol) in ethanol (350 mL) were heated to reflux for 2 h. After completion of the reaction by TLC, the reaction mixture was evaporated to dryness. The semi-pure compound obtained was partitioned between diethyl ether and cold water. All the combined organic layers were washed with brine solution and dried over anhydrous Na_2SO_4. After filtration and removal of the volatiles under reduced pressure, the residue obtained was purified by flash column chromatography (silica-gel, 100-200 mesh) using chloroform/methanol (8:2) as an eluent to afford compound **4** (12.36 g, 63% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (b s, 1H), 7.84 (d, *J*=7.8 Hz, 1H), 7.46 - 7.37 (m, 2H), 7.29 - 7.20 (m, 1H), 5.95 (s, 2H), 4.31 (b s, 2H); LC/MS (ESI): m/z 192.24 $[M+H]^{+}$; FT-IR (KBr) 3261 (NH), 3108(NH_2),1676 (C=O) cm^{-1}.

General procedure for the synthesis of 3-amino-N'-(3,4-dichloro benzylidene) benzofuran-2-carbohydrazide (5)

To a stirred suspension of 3-amino-benzofuran-2-carbohydrazide **4** (3 g, 15.69mmol), 3,4-dichloro benzaldehyde (3.02 g, 17.26 mmol), glacial acetic acid (0.941 g, 15.69 mmol) in absolute ethanol (60 mL) were heated to reflux for 2 h. The reaction mixture was evaporated to dryness. The semi-pure compound obtained was partitioned between diethyl ether and cold aq $NaHCO_3$ solution. All the combined organic extracts were washed with brine solution and dried over anhydrous sodium sulphate. After filtration and removal of the volatiles under reduced pressure, the residue obtained was purified by flash column chromatography (silica-gel, 100-200 mesh) using petroleum ether/ethyl acetate (6:4) as an

eluent to afford compound **5** (2.86 g, 52.5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ11.80 (s, 1H), 8.44 (s, 1H), 8.00 - 7.89 (m, 2H), 7.75 - 7.65 (m, 2H), 7.49 (d s, 2H), 7.29 (dd, *J*=2.8, 5.2, 7.8 Hz, 1H), 6.36 (s, 2H); MS, m/z 348.30 $(M+H)^+$, m/z 350.32 $(M+H)^+$.

Confirmation of Regioselectivity of compound (5)

The HMBC and HSQC spectroscopic data confirms the introduction of hydrazone regioselectivity of amine/hydrazide with aldehyde to form hydrazone was further conformed based on the NOESY spectrum. And it is clearly evident the absence of imine proton correlation with benzofuran phenyl ring protons and we concluded the substitution of 3,4-dichloro phenyl hydrazone is on 02nd position of benzofuran like compound **5** (Fig-4).

General procedure for the synthesis of sulphonamide compounds (6a-6b)

4-bromo-N-(2-(2-(3,4-dichlorobenzylidene)hydrazinecarbonyl)benzofuran-3-yl)benzene sulfonamide (6a)

To a stirred solution of 3-amino-N'-(3,4-dichlorobenzylidene)benzofuran-2-carbohydrazide **5** (100 mg, 0.288 mmol) in THF (0.5 mL) was added 4-bromobenzene-1-sulfonyl chloride (109 mg, 0.432 mmol) at 0°C followed by DIPEA (88 mg, 0.72 mmol) and DMAP (12 mg, 0.098 mmol), the reaction mixture was allowed to stir at room temperature for 16 h. After completion of the reaction by TLC, the reaction mixture was concentrated to afford residue which was purified by reverse phase preparative chromatography (Grace C-18 column, 0.1% formic acid in water and ACN as mobile phase) the fraction was lyophilised to afford **6a** (16 mg, 0.028 mmol, 9% yield) as white solid. Melting range (°C) 238-241 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.32 (br s, 1H), 10.18 (br s, 1H), 8.26 (br s, 1H), 8.03 - 7.85 (m, 1H), 7.81 - 7.41 (m, 7H), 7.32 (br s, 1H); MS, $C_{22}H_{15}Cl_2N_3O_4S$, observed formula weight is 487.0160, found m/z 488.24 $(M+H)^+$, its isotope m/z 490.28 $(M+H+2)^+$.

N-(2-(2-(3,4-dichloro benzylidene) hydrazinecarbonyl)benzofuran-3-yl)-4-methylbenzene sulfonamide (6b)

12 mg of **6b** (0.0239 mmol, 8% yield) as yellow solid. Melting range (°C) 168-170 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.08 (br s, 1H), 8.25 - 8.15 (m, 1H), 7.94 (br d, *J* = 8.1 Hz, 1H), 7.87 - 7.82 (m, 1H), 7.76 - 7.62 (m, 3H), 7.47 - 7.40 (m, 1H), 7.32 (br t, *J* = 7.7 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.11 (br t, *J* = 7.5 Hz, 1H), 2.26 (s, 3H); HRMS, $C_{23}H_{17}Cl_2N_3O_4S$, observed formula weight is 501.0317, found m/z 502.0391 $(M+H)^+$, its isotope m/z 504.0391 $(M+H+2)^+$.

General procedure for the synthesis of amide compounds (7a-7d) 4-bromo-N-(2-(2-(3,4-dichlorobenzylidene) hydrazinecarbonyl)benzofuran-3-yl) benzamide (7a)

To a stirred solution of 3-amino-N'-(3,4-dichlorobenzylidene)benzofuran-2-carbohydrazide **5** (100 mg, 0.288 mmol) in THF was added 4-bromobenzoic acid (86 mg, 0.432 mmol) at 0 °C followed by T_3P (50% in EtOAc, 0.4 mL) DiPEA (88 mg, 0.72 mmol), the reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was diluted with ethyl acetate (25 mL) and washed with water and brine solution. All the combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated to afford semi-pure compound. This was purified by reverse phase preparative HPLC (Grace C-18 column, 0.1% formic acid in water and ACN as mobile phase), the fraction was lyophilised to afford **7a** (18 mg, 0.034 mmol, 11% yield) as white solid. Melting range (°C) 228 - 230 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.53 (br s, 1H), 10.91 (br s, 1H), 8.53 (s, 1H), 8.24 (s, 1H), 8.13 - 8.01 (m, 2H), 7.97 (s, 1H), 7.90 (br d, *J*=7.7 Hz, 1H), 7.80 - 7.66 (m, 3H), 7.59 (br t, *J*=7.9 Hz, 2H), 7.41 (br t, *J*=7.7 Hz, 1H); HRMS, $C_{23}H_{14}BrCl_2N_3O_3$, observed formula weight is 528.9596, found m/z 529.9647 $(M+H)^+$, its isotope m/z 531.9647 $(M+H+2)^+$; FT-IR (KBr) 3422 (NH), 3261 (NH), 1678 & 1620 (C=O) cm^{-1}.

N-(2-(2-(3,4-dichlorobenzylidene) hydrazinecarbonyl)benzofuran-3-yl)-4-methylbenzamide (7b)

18 mg of **7b** (0.034 mmol, 11% yield) as white solid. Melting range (°C) 239 - 241 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.55 (br s, 1H), 10.93 (br s, 1H), 8.54 (s, 1H), 8.28 (br d, *J*=7.7 Hz, 1H), 8.05 - 7.87 (m, 3H), 7.80 - 7.66 (m, 3H), 7.60 (br t, *J*=7.7 Hz, 1H), 7.49 - 7.34 (m, 3H), 2.47 - 2.35 (s, 3H); HRMS, $C_{24}H_{17}Cl_2N_3O_3$ observed formula weight is 465.0647, found m/z 466.0698 $(M+H)^+$, its isotope m/z 468.0698 $(M+H+2)^+$; FT-IR (KBr) 3450 (NH), 3226 (NH), 1665, 1646 & 1620 (C=O) cm^{-1}.

N-(2-(2-(3,4-dichlorobenzylidene) hydrazinecarbonyl) benzofuran-3-yl)-3,4-dimethoxybenzamide (7c)

12 mg of **7c** (0.0234 mmol, 8% yield) as white solid. Melting range (°C) 238 - 240 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.54 (br s, 1H), 10.87 (br s, 1H), 8.54 (s, 1H), 8.22 (br d, *J*=7.3 Hz, 1H), 7.97 (s, 1H), 7.80 - 7.52 (m, 6H), 7.39

(br t, *J*=7.7 Hz, 1H), 7.18 (br d, *J*=8.4 Hz, 1H), 3.88 (s, 6H); HRMS, $C_{25}H_{19}Cl_2N_3O_5$ observed formula weight is 511.0702, found m/z 512.08 $(M+H)^+$, its isotope m/z 514.082 $(M+H+2)^+$; FT-IR (KBr) 3462 (NH), 3302 (NH), 3226 (NH), 2913 (CH_3), 1681 and 1617 (C=O) cm^{-1}.

N-(2-(2-(3,4-dichlorobenzylidene) hydrazinecarbonyl)benzofuran-3-yl)-3,4,5-trimethoxybenzamide (7d)

10 mg of **7d** (0.0184 mmol, 6% yield) as white solid. Melting range (°C) 276 - 278 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (s, 2H), 8.10 (br s, 1H), 7.93 (br s, 1H), 7.80 - 7.62 (m, 3H), 7.53 (br t, *J*=7.5 Hz, 1H), 7.44 - 7.30 (m, 3H), 3.89 (br s, 6H), 3.77 (s, 3H); HRMS, $C_{26}H_{21}Cl_2N_3O_6$ observed formula weight is 541.0807, found m/z 542.0853 $(M+H)^+$, its isotope m/z 544.0853 $(M+H+2)^+$; FT-IR (KBr) 3454 (broad, NH), 2938 (CH_3), 1643 and 1614 (C=O) cm^{-1}.

General procedure for the synthesis of urea compounds (8a and 8b)

N'-(3,4-dichlorobenzylidene)-3-((((3,4-dimethoxyphenyl)imino)methylene)amino) benzofuran-2-carbohydrazide (8a)

To a stirred solution of 3-amino-N'-(3,4-dichlorobenzylidene)benzofuran-2-carbohydrazide **5** (100 mg, 0.288 mmol) in THF was added 3,4-dimethoxy phenyl thioisocyanate (112 mg, 0.574 mmol) at 0 °C followed by DiPEA (129 µL, 0.72 mmol), and DMAP (12 mg, 0.098 mmol).The reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was concentrated to afford residue which was purified by reverse phase preparative chromatography (Grace C-18 column, 0.1% formic acid in water and ACN as mobile phase) to afford **8a** (21 mg, 0.028 mmol, 14% yield) as off-white solid. Melting range (°C) 296 - 298 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.51 (br s, 1H), 7.94 (br d, *J*=7.3 Hz, 1H), 7.88 - 7.53 (m, 6H), 7.52 - 7.38 (m, 2H), 7.05 - 6.90 (m, 2H), 6.75 (br d, *J*=9.2 Hz, 1H), 3.76 (d, *J*=14.7 Hz, 6H)); MS, $C_{25}H_{18}Cl_2N_4O_4$ observed formula weight is 508.0705, found m/z 509.38 $(M+H)^+$, its isotope m/z 511.40 $(M+H+2)^+$; FT-IR (KBr), 3448 (broad, NH), 3357 (NH), 2956, 2827 (CH_3), 1689 and 1599 (C=O) cm^{-1}.

N'-(3,4-dichlorobenzylidene)-3-((((3,4,5-trimethoxyphenyl)imino)methylene) amino)benzofuran-2-carbohydrazide (8b)

21 mg of **8b** (0.028 mmol, 14% yield) as an off-white solid. Melting range (°C) 254 - 256 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.98 (br d, *J*=7.7 Hz, 1H), 7.86 (d, *J*=1.8 Hz, 1H), 7.77 (br t, *J*=8.3 Hz, 2H), 7.69 - 7.59 (m, 2H), 7.51 - 7.43 (m, 2H), 7.14 (s, 2H), 6.91 (br d, *J*=9.2 Hz, 1H), 3.84 (m, 6H), 3.62 (m, 3H); HRMS, $C_{26}H_{20}Cl_2N_4O_5$ observed formula weight is 538.0811, found m/z 539.09 $(M+H)^+$, its isotope m/z 541.14 $(M+H+2)^+$; FT-IR (KBr), 3373 (NH), 3068 (NH), 2961, 2930, 2828 (CH_3), 1704, 1612 (C=O) and 1596 (C=N)cm^{-1}.

General procedure for the synthesis of urea compounds (9a-9c)

1-(3-chlorophenyl)-3-(2-(2-(3,4-dichlorobenzylidene)hydrazinecarbonyl) benzofuran-3-yl)urea (9a)

To a stirred solution of 3-amino-N'-2-(3,4-dichlorobenzylidene)benzofuran-2-carbohydrazide **5** (100 mg, 0.288 mmol) in THF was added 3-chloro phenyl isocyanate (86 mg, 0.432 mmol) at 0 °C followed by DiPEA (129 µL, 0.72 mmol), and DMAP (12 mg, 0.098 mmol). The reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was concentrated to afford residue which was purified by reverse phase preparative chromatography (Grace C-18 column, 0.1% formic acid in water and ACN as mobile phase) to afford **9a** (14 mg, 0.028 mmol, 9% yield) as white solid. Melting range (°C) 281 - 283 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.40 (br s, 1H), 10.26 (br s, 1H), 9.63 (br s, 1H), 8.60 - 8.45 (s, 1H), 8.21 (br d, *J*=8.1 Hz, 1H), 7.96 (s, 1H), 7.84 - 7.70 (m, 3H), 7.68 - 7.61 (m, 1H), 7.60 - 7.51 (m, 1H), 7.43 - 7.25 (m, 3H), 7.07 (br d, *J*=3.3 Hz, 1H)); HRMS, $C_{23}H_{15}C_{l3}N_4O_3$ observed formula weight is 500.0210, found m/z 501.0263 $(M+H)^+$, its isotope m/z 503.0268 $(M+H+2)^+$; FT-IR (KBr), 3490 (NH), 3330, 3229 (NH), 3073 (C=C), 2918, 2850 (CH), 1687, 1622 (C=O), and 1590 (C=N) cm^{-1}.

1-(2-(2-(3,4-dichlorobenzylidene)hydrazinecarbonyl)benzofuran-3-yl)-3-phenylurea (9b)

16 mg of **9b** (0.0343 mmol, 12% yield) as white solid. Melting range (°C) 285 - 287 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 12.40 (s, 1H), 9.97 (br s, 1H), 9.43 (s, 1H), 8.62 - 8.46 (m, 1H), 8.22 (br d, *J*=8.1 Hz, 1H), 7.96 (s, 1H), 7.75 (s, 2H), 7.67 - 7.43 (m, 4H), 7.34 (q, *J*=7.6 Hz, 3H), 7.07 - 6.95 (m, 1H); HRMS, $C_{23}H_{16}Cl_2N_4O_3$ observed formula weight is 466.0599, found m/z 467.0650 $(M+H)^+$, its isotope m/z 469.0650 $(M+H+2)^+$; FT-IR (KBr), 3327 (NH), 3226 (NH), 3059 (C=C), 2917, 2851 (CH), 1685, 1645, 1625 (C=O), and 1592 (C=N) cm^{-1}.

1-(2-(2-(3,4-dichlorobenzylidene) hydrazinecarbonyl)benzofuran-3-yl)-3-(2-methoxy-6-methylphenyl)urea (9c)

12 mg of **9c** (0.0235 mmol, 8% yield) as white solid. Melting range (°C) 302 - 304 °C; ^{1}H

NMR (300 MHz, DMSO-d_6) δ 12.32 (br s, 1H), 9.68 (br s, 1H), 9.21 (br s, 1H), 8.79 (br s, 1H), 8.53 (br s, 1H), 8.07 - 7.83 (m, 3H), 7.79 - 7.53 (m, 3H), 7.34 (br t, *J*=7.5 Hz, 1H), 6.99 - 6.77 (m, 2H), 3.87 (br d, *J*=11.7 Hz, 3H), 2.24 (s, 3H); HRMS, $C_{25}H_{20}Cl_2N_4O_4$ observed formula weight is 510.0862, found m/z 511.0938 $(M+H)^+$, its isotope m/z 513.0931 $(M+H+2)^+$.

Anti-microbial activity

The antimicrobial activity of the 3-amino-benzofuranhybrids were determined using well diffusion method[11] against different pathogenic reference strains procured from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded on the surface of the Muller-Hinton agar Petri plates, with 0.1 mL of previously prepared microbial suspensions individually containing 1.5 x 10^8 cfu $mL^{-}1$ (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the medium plates using a corkborer and the synthesized Benzofuran 1,3,4-oxadiazole hybrids at a dose range of 125 - 0.97 µg $well^{-1}$ was added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solution of Ciprofloxacin at a dose range of 125 - 0.97 µg $well^{-1}$ served as positive control, while the well containing DMSO served as negative control. The plates were incubated for 24 h at 37 °C and the well containing the least concentration showing the inhibition zone was considered as MIC. All experiments were carried out in duplicates and mean values are represented.

Similarly antifungal activity of the synthesized 3-amino-benzofuran hybrids were determined using well diffusion method[11] against one fungal strain to understand the preliminary response was used Candida *albicans* (MTCC 3017) and it was procured from the Wells of 6.0 mm diameter were prepared in the media plates using a corkborer and the synthesized compounds dissolved in 10% DMSO at a dose range of 125 - 0.97 µg/mL were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solutions of Miconazole at a dose range of 125 - 0.97 µg $well^{-1}$, served as positive control, while the well containing DMSO served as negative control. The plates were incubated for 24 h at 30 °C for different Candida strains. The well containing the least concentration showing the inhibition zone is considered as the minimum inhibitory concentration. All the experiments were carried out in duplicates and mean values are represented.

***Mycobacterium Tuberculosis* Chorismate Mutase (*MtbCM or CM) inhibition activity**

All the synthesized 3-amino-benzofuran derivatives were tested for their inhibitory potential against *Mycobacterium tuberculosis* H37Rv chorismate mutase (CM). The assay[12,13] involved determination of activity of enzyme CM which catalyzes the conversion of chorismate to prephenate. Thus determination of activity of CM is based on the direct observation of conversion of chorismic acid to prephenate spectrophotometrically at OD274. This reaction was performed in the presence of test compounds to determine their CM inhibiting activities. A known inhibitor of CM i.e. 4-(3,5-dimethoxyphenethylamino)-3-nitro-5-sulfamoylbenzoic acid[14] was prepared and used as a reference compound the IC_{50} value of which was found to be less than 10 µM. *Mycobacterium tuberculosis* chorismate mutase (MtCM) gene was PCR amplified and cloned into expression vector pET22b. MtbCM was purified from over expressed culture of BL21 (DE3) harboring pET22b/ MtbCM by Ni-NTA affinity chromatography. Activity of chorismate mutase enzyme is based on the direct observation of conversion of chorismate to prephenate Spectrophotometrically at OD_{274}. The reaction volume of 100 µL contained 50 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 0.1 mg/mL bovine serum albumin, and 10 mM β-mercaptoethanol, and chorismic acid 4 mM. The reaction was started by adding 180 pmol of purified protein to the pre-warmed chorismic acid solution. Inhibitory screening of the test compounds against chorismate mutase activity was measured at 30 µM concentration of the effectors. The reaction was allowed to proceed at 37 °C and was terminated after 5 min with 100 µL of 1 N HCl. A blank with no enzyme for every reaction was kept as a control to account for the non enzymatic conversion of chorismate to prephenate. The percentage of enzyme inhibition caused by the test compound is calculated by the following formula (% inhibition = 100 – residual activity of CM).

3. RESULTS AND DISCUSSION

Chemistry

Molecular hybridization to identify a new lead compound was achieved by isolation of 3-amino-benzofuran derivatives and all the final compounds synthesized were isolated using SiO_2 chromatography and characterized by using spectroscopic analysis. Compound **5** regioselectivity was confirmed by HMBC, HSQC and NOESY experiments where the

imine proton interactions were clearly absent with benzofuran ring protons. So the primary amine at 03rd position of benzofuran was substituted with selected sulphonamides, benzamides and urea derivatives based on the best glide score obtained through docking studies with Chorismate Mutase protein. Keeping the 2,3-disubstitutions on benzofuran the research can be extended to find the more suitable lead compounds for CM inhibition.

Anti-Bacterial activity

All the isolated compounds were screened for *in vitro* antibacterial activity[11,15,16] against different Gram-positive and Gram-negative bacterial strains. Among all the benzofuran derivatives screened, compound **7d** have showed promising growth inhibition of gram +ve and –ve bacteria (MIC values for gram +ve bacteria is 3.9 to 15.6 µg/mL, whereas with gram –ve bacteria is 15.6 µg/mL). Most of the compounds exhibited better selectivity towards gram +ve bacteria. Among the many pathogenic Candida species, most notably Candida *albicans* is a dimorphic fungus behaving as a commensal or an opportunistic pathogen causing both superficial and systemic infections. Among the tested compounds no one are found to have inhibition activity against *Candida albicans*(MTCC 3017). Compounds **7b, 7c** and **7d** are the benzamides derivatives have increased substitution on phenyl ring have showed better inhibition of gram+ve bacteria but not of gram-ve bacteria. Sulphonamides **6a** & **6b** are less probably contributing to the antimicrobial activity. Urea derivatives compound **9b** and **9c** are found to have moderate growth inhibition of microbial strains. No one of the tested compounds has showed growth inhibition of fungal strain. Antimicrobial activity and Chorismate Mutase inhibition of target compounds results to this regard are tabulated in Table 1.

***Mycobacterium Tuberculosis* Chorismate Mutase inhibition activity**

Compounds **6a**, **6b**, **7b, 7c, 7d, 9a, 9b** and **9c** are found to have >50% inhibition of CM compared to other molecules when tested at 30 µM (Table 1) whereas rest of the compounds were either less active or inactive. Notably, compound **7d (**3,4,5-trimethoxy benzamides substitution) have showed extensively potent against Chorismate Mutase (84%). Thus N-(2-(2-(3,4-dichlorobenzylidene)hydrazinecarbonyl)benzofuran-3-yl)-3,4,5-trimethoxybenzamide framework appeared as a new scaffold for the development of novel inhibitors of Chorismate Mutase. Since tuberculosis is a leading cause of death worldwide, the present classes of compounds are of further interest as potential anti-tubercular agents. All these isolated compounds will be screened against different types of *Mycobacterium Tuberculosis* strains.

4. CONCLUSION

A series of novel 3-benzamide-Benzofuran-2-hydrazone analogues (**6a-6b, 7a-7d, 8a, - 8b, 9a-9c)** were synthesized from 3-amino-N'-(3,4-dichlorobenzylidene)benzofuran-2-carbohydrazide **5** and screened against gram+ve, -ve bacteria and Chorismate Mutase inhibition. Among the screened compounds **7d** was exhibited maximum growth inhibition on gram+ve bacteria (MIC values 3.9 – 7.8 µg/mL) and Chorismate Mutase inhibition (84%) as well**.** None of these compounds have showed antifungal activity against Candida *albicans*. Compound **7d** have showed promising inhibitory activities when tested at 30 µM. Overall; this research has identified 3-amino-benzofuran-2-hydrazone as a new scaffold for the development of new inhibitors of Chorismate Mutase. In conclusion, we have described the design, synthesis and *in vitro* evaluation of novel N-(2-(2-(3,4-dichlorobenzylidene)hydrazinecarbonyl)benzofuran-3-yl)-3,4,5-trimethoxybenzamide **7d** and analogues as potential inhibitors of gram +ve bacteria and for Chorismate Mutase inhibition.

ACKNOWLEDGEMENTS

Authors are grateful to GVKBIO sciences Pvt. Ltd. for the financial support and for providing laboratory facilities. We specially thank to Dr. Ravikumar. P, Dr. Krishna. Ethiraj and Dr. Sudhir Kumar Singh for their scientific inputs, moral support, and motivation throughout my Ph.D. research work. We also thank to our analytical department for assistance in getting the spectral data. We specially acknowledge Dr. Manojith. Pal for his kind support during the Chorismate Mutase (CM) inhibition activity assay.

Disclosure statement

The authors declare no conflicts of interests.

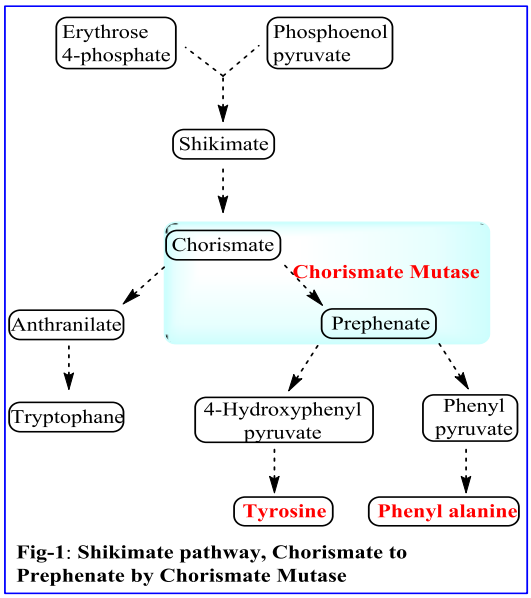


Fig-1: **Shikimate pathway, Chorismate to Prephenate by Chorismate Mutase**

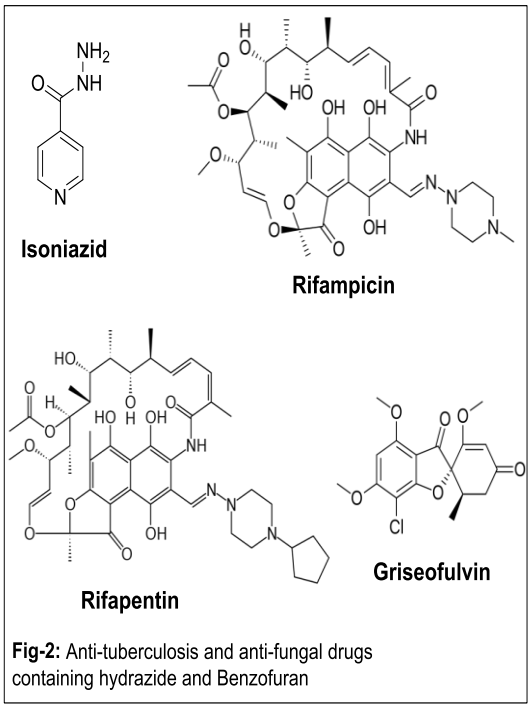


Fig-2: Anti-tuberculosis and anti-fungal drugs containing hydrazide and Benzofuran

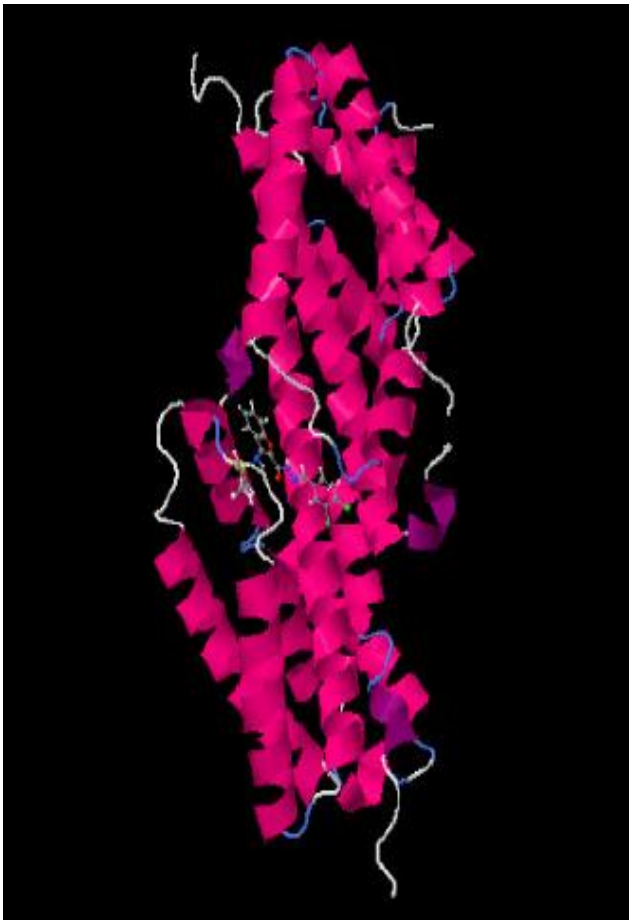


Fig. 3: Docking of Compound 6b into Chorismate Mutase (*MtbCMor CM, PDS code 2FP2) using Swiss Dock software

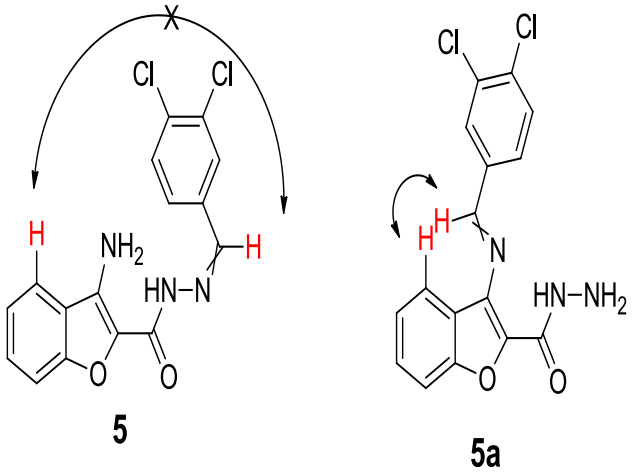


Fig-4: HMBC, HSQC & NOESY experimets to conform the structure of compound **5**

Table 1: Antimicrobial activity and Chorismate Mutase inhibition of target compounds

Test Compounds	Minimum inhibitory concentration (µg/mL)								CM % inhibition @ 30 µM
	Gram+ve bacteria					*Gram-ve bacteria*		*Fungi*	
	Staphylococcus aureus **(MTCC 96)**	***Bacillus subtilis*** **(MTCC 121)**	***Staphylococcus aureus*** **(MLS16 MTCC 2940)**	***Micrococcus luteus (MTCC 2470)***	***Klebsiella planticola*** **(MTCC 530)**	***Escherichia coli*** **(MTCC 739)**	***Pseudomonas aeruginosa*** **(MTCC 2453)**	***Candida albicans*** **(MTCC 3017)**	
5	15.6	62.5	>125	>125	>125	>125	>125	>125	41
6a	7.8	15.6	62.5	15.6	>125	>125	>125	>125	51
6b	31.2	15.6	>125	>125	>125	>125	>125	>125	56
7a	62.5	31.2	3.9	>125	>125	>125	>125	>125	48
7b	>125	62.5	31.2	>125	62.5	>125	>125	>125	62
7c	62.5	3.9	7.8	62.5	62.5	62.5	62.5	62.5	64
7d	7.8	3.9	15.6	7.8	15.6	15.6	62.5	>125	**84**
8a	>125	>125	31.2	>125	15.6	>125	>125	>125	31
8b	>125	>125	62.5	>125	62.5	>125	>125	>125	26
9a	62.5	62.5	>125	62.5	>125	62.5	>125	>125	52
9b	62.5	>125	62.5	15.6	62.5	>125	62.5	>125	63
9c	>125	>125	31.2	>125	62.5	>125	62.5	>125	58
Ciprofloxacilin e	0.9	0.9	0.9	0.9	0.9	0.9	0.9	N	100
Miconazole	N	N	N	N	N	N	N	7.8	N
4-(3,5-dimeth-oxyphenethyl amino)-3-nitro-5-sulfamoylben zoic acid	N	N	N	N	N	N	N	N	99

Where control is DMSO, N= not applicable
Results represented the average values of two independent experiments.

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