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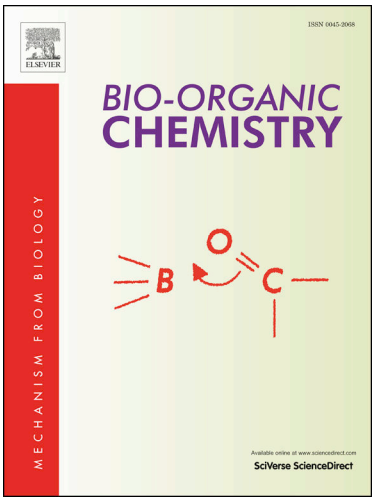


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# Design, Synthesis, *In vitro* and In silico Studies of 2, 3-diaryl Benzofuran Derivatives as Antitubercular agents

Balakishan Bhukya[a], Aparna Shukla[b], Vinita Chaturvedi[c], Priyanka Trivedi[c], Shailesh Kumar[d], Feroz Khan, Arvind S. Negi[a]* and Santosh Kumar Srivastava[a]*

[a]*Medicinal Chemistry Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India-226015,* [b]*Metabolic & Structural Biology Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow-226015,* [c]*Biochemistry Division, Central Drug Research Institute, Lucknow, India-226001,* [d]*Department of Applied Chemistry, Babasaheb Bhimrao Ambedkar University, Lucknow-India 2260*

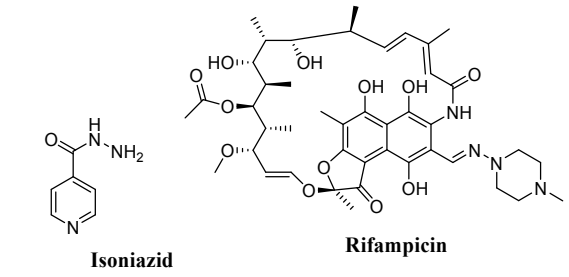
*Address correspondence Medicinal Chemistry Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India-226015; Tel: +91-522-2718581; Fax: +91-522-2342666; E-mail: skscimap@gmail.com CIMAP publication No.: CIMAP/PUB/2020/MAR/14

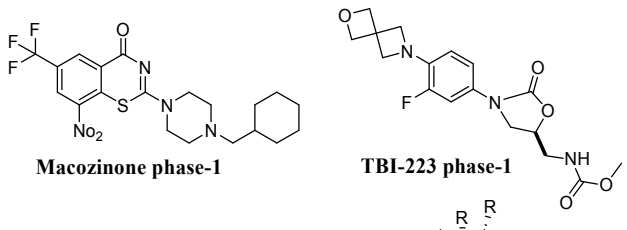
**Abstract:** As a part of our drug discovery program for anti-tubercular agents, a total of seventeen 2, 3-diaryl benzofuran hybrids were designed, synthesized and screened for their anti-tubercular potential against *Mycobacterium tuberculosis* H37Ra avirulent strain. Out of seventeen, four derivatives showed significant activity against *M.tuberculosis* H37Ra Virulent strain (ATCC 25177) with MIC value ranging from 12.5-50 µg/mL But out of four, one derivative (**9E**) was significantly active (MIC 12.5ug/mL), which was further supported by the molecular docking score (-8.4) with respect to the first line anti-tubercular drug, isoniazid (-6.2) on the target Polyketide Synthase-13. All the derivatives were also evaluated for their cytotoxicity against the normal lung cell line L-132 by the MTT assay and no toxicity was observed up to 27.4 µg/mL concentration. This report on the antitubercular potential of benzofuran derivatives may be of great help in anti-tubercular drug development.

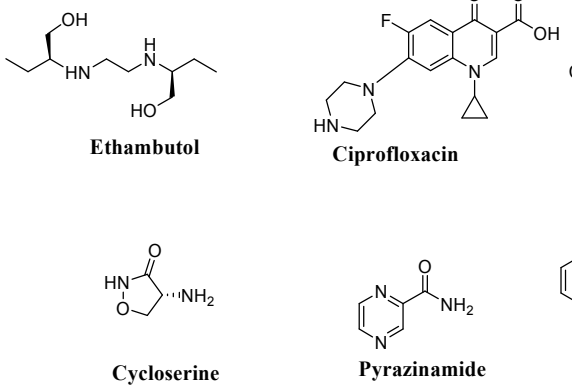
**Keywords:** Benzofuran, semi-synthetic derivatives, anti-tubercular activity, i*n-silico* studies.

## INTRODUCTION

Tuberculosis (TB) is a contagious air-borne bacterial disease caused by the infection of *Mycobacterium tuberculosis* (MTB), which mainly affects the lungs. According to global tuberculosis, report-2018 Tuberculosis (TB) is one of the top 10 causes of death worldwide in 2017. It is also the leading killer of people with HIV and a major cause of death related to antimicrobial resistance. TB is treated with drugs as given in Figure 1 with a complex treatment regimen, consisting of multiple antibiotics that target diverse cellular processes. In 2017 ten million new TB cases were reported worldwide mainly affecting men 5.8 million, women 3.2 million and children's 1 million. In 2017 1.6 million people were died from Tubercular disease, including 0.3 million among people with HIV. Over a period of time, the pathogen has become resistant to the two most powerful drugs like rifampicin and fluoroquinolones. Globally in 2017, 558000 people developed TB cases that were resistant to rifampicin (RR-TB) and 160000 cases of Multidrug resistance as well as rifampicin resistance (MDR-TB/ RR-TB). Although, there are several antitubercular drug molecules in pipeline as shown in Figure 2, but to tackle this situation, still there is an urgent need to explore new anti-TB molecules [**1, 2**]. Natural products play a major role in the discovery of new drug leads as they are having novel structures, excellent biological activities and absolute selectivity. Almost 50% of the FDA approved drugs are either the natural products or natural product derivatives [**3, 4, 5**].



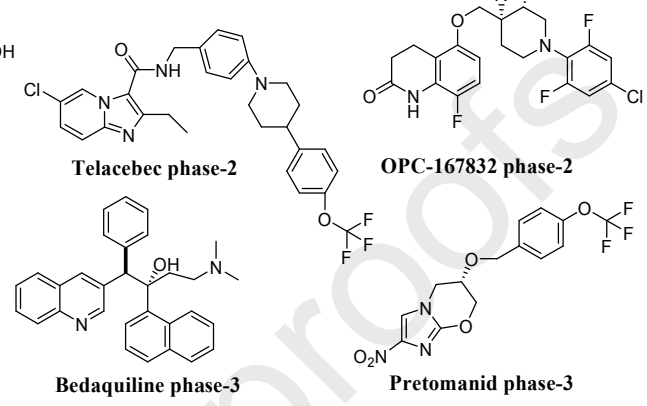




**Figure-1: Anti-tubercular drugs already in the market**

**Figure-2**: **New anti-tubercular drugs in pipeline**

It has been reported that naturally isolated flavonoids possess 37.78% more antitubercular activity than any other class of natural compounds. In this regard benzofuran derivatives have shown versatile biological activities (not less than 47 types of activities [**6**] and some of the benzofuran derivatives are already in the market (Rifampicin) while few are in the pipelines. Recently it has been reported that benzofuran [**7**] pharmacophore is of significant importance in the development of antitubercular drugs [**8**]. Hence, as a part of our drug discovery program for anti-tubercular agents [**9**-**14**], design and synthesis of three 2,3-di aryl benzofuran prototypes (Figure 3) was carried out and all the derivatives were *in-vitro* evaluated for their antitubercular potential against Mtb H37Ra strain. Further, *in-silico* and cytotoxicity studies were also carried out for the most active derivatives.

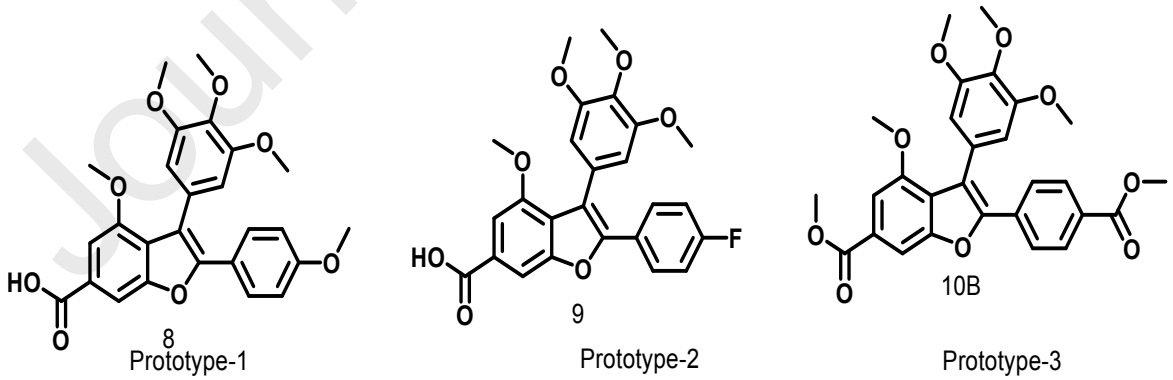



**Figure (3)**: Designed prototypes

**GENERAL EXPERIMENT PROCEDURE**

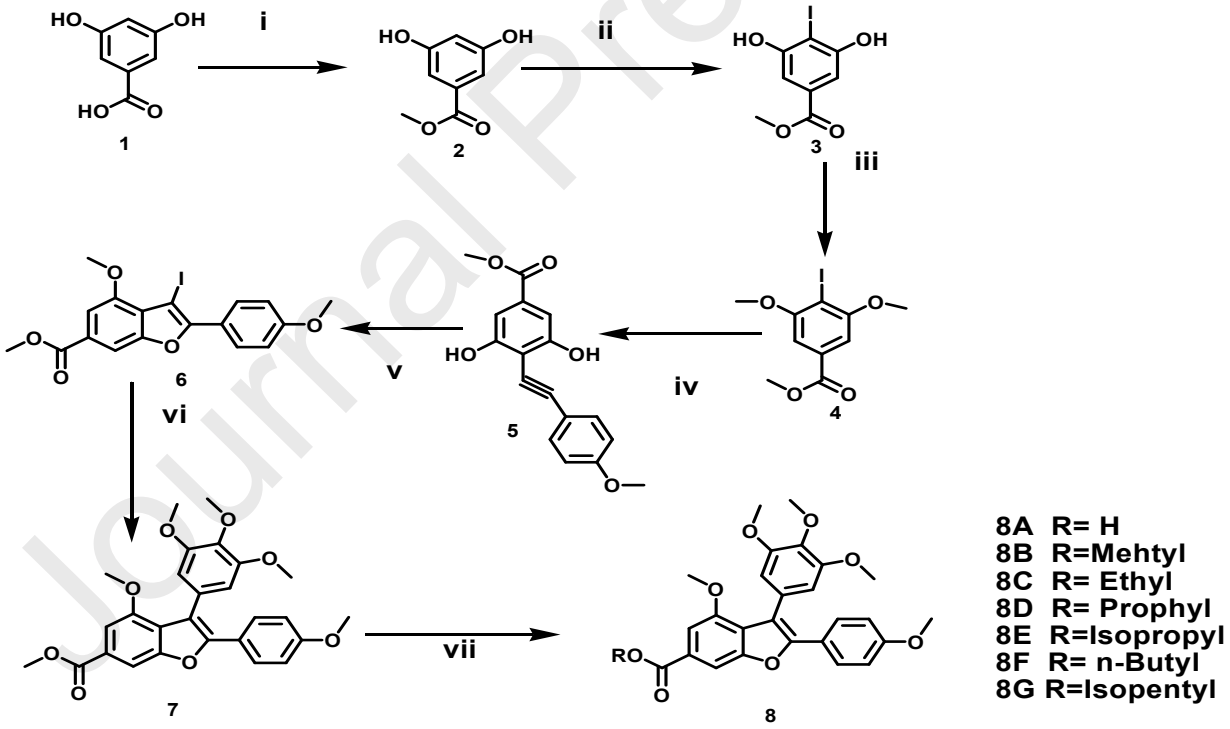
Melting points were determined on E-Z Melt automated melting point apparatus (Stanford Research System, USA) in open glass capillaries and were uncorrected. Reagents and other chemicals were procured from Merck India Limited, Avra Synthesis, India and used without purification. Reactions were monitored on Merck pre-coated silica gel TLC-$GF_{254}$ aluminum sheets and detection was done under UV cabinet (254nm and 365nm) and Compounds were purified over silica gel (60-120 and 100-200 mesh) and characterized by $^{1}H$ and $^{13}C$ NMR, ESI-MS, and ESI-HRMS. The NMR spectra were obtained on Bruker Avance-500 MHz Spectrometers. Chemical shifts are given in δ ppm values with tetramethylsilane (TMS) as reference. $^{1}H$–$^{1}H$ coupling constant (J) values are given in Hz. ESI mass spectra were recorded on Shimadzu LC-MS and High-Resolution Mass (HRMS) on Agilent 6545 Q-TOF after dissolving the compounds in methanol.

**Synthesis of 2, 3-di aryl benzofuran prototypes:**

The synthesis of 2, 3-di aryl benzofuran prototypes such as prototype-1, prototype-2 and prototype-3 were synthesized as shown in scheme-1, scheme-2 and scheme-3.

**Scheme-1**

The scheme-1 was started with 3', 5'- dihydroxybenzoic acid-**1** as a starting material. Then compound-**1** methylated with concentrated sulphuric acid in methanol at $80^{C}$ temperature to get 2. Then 2 was iodinated with N-iodo succinimide performed in methanol to get **3**.




**Scheme-1**-Reagent and condition: (i) Conc-$H_2SO_4$, methanol, temp $80^{o}C$, 4h, 98%; (ii) NIS, methanol, rt, 16h, 98%: (iii) $K_2CO_3$, methyl iodide, DMF, rt, 15h 98% : (iv) $(Ph_3P)_2PdCl_2$, CuI, aryl iodide, triethylamine, 60 ˚C, 5h, 98%: (v) iodine, $CH_2Cl_2$, rt, 23h, 99%: (vi) Trimethoxy phenyl boronic acid, $NaHCO_3$, $(Ph_3P)_2PdCl_2$, DMF/$H_2O$ (4:1), $80^{o}C$, 7h, 96%: (vii) KOH, MeOH/ $H_2O$ (9:1), $80^{o}C$, 6h, 98%.

Then methylation has carried out in the presence of potassium carbonate and methyl iodide in DMF to get compound-**4**. By using compound-**4** and 4-methoxy phenylacetylene exploiting the reaction of Sonogashira cross-coupling reaction afforded compound -**5**. But Sonogashira carbon-carbon bond formation reaction, aryl halides which contain electron-donating groups have given higher yields and electron-withdrawing groups were provided lower yields. The iodo cyclization of compound-5 was done at rt by using molecular iodine as a reagent to get 6. With the compound-**6** and 3', 4', 5'- Trimethoxy phenylboronic acid in the presence of Suzuki-cross coupling reaction to getting **7,** which was demethylated in presence of 10% KOH in methanol and water (9:1) to get final pharmacophore **8**.

**Synthesis of Methyl 3, 5-dihydroxybenzoate (2): -** The reaction was started with 3, 5-dihydroxybenzoic acid (1g, 0.00649 mol), in methanol (10 mL), and concentrated sulfuric acid (96% pure in water, 0.5 ml, 0.0093 mmol) slowly added to the above solution. The reaction mixture was heated to reflux for 3h. The reaction mixture was cooled to room temperature and then solvent removed under reduced pressure. The residue was extracted with ethyl acetate (50mL) and washed with water (10mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated. The crude residue so obtained was purified by column chromatography (hexane-ethyl acetate 14%), which afforded compound **2** as a white solid in 94% yield.

**Synthesis of Methyl 3,5-dihydroxy-4-iodobenzoate (3):-** To a stirred solution of methyl 3, 5-dihydroxybenzoate (250 mg, 1.488 mmol) in methanol (1 mL) N-iodosuccinamide (351.5 mg, 1.57 mmol) was added at 0 $^0$C. After stirring at room temperature for 16 h, the reaction mixture was poured into Ice-cold water (5mL) and then this solution was transferred into a separatory funnel and slowly saturated aqueous solution of sodium thiosulfate (5 mL) was added to it, which after 5 minutes resulted in the formation of a white solid compound. The mixture was extracted thrice with EtOAc (5-7 mL). The combined organic layer was dried over $Na_2SO_4$ and solvent removed on rotary evaporator, which afforded the crude product-**3**.

**Synthesis of Methyl 4-iodo-3, 5-dimethoxybenzoate (4): -** To a stirred solution of methyl 3, 5-dihydroxy-4-iodobenzoate (230 mg, 0.78 mmol) in DMF (1.5 mL) $K_2CO_3$ (386.5 mg, 3.9 mmol) was added at rt. After 20 min of stirring at room temperature, methyl iodide (0.15 mL, 2.35 mmol) was added and the resulting solution was further stirred for 15 h at RT. After completion of the reaction, the mixture was extracted thrice with EtOAc. The combined organic layer was dried with $Na_2SO_4$ and concentrated on rotary evaporator. The crude so obtained was purified by column chromatography, which afforded a half white compound-**4** (230mg, 81%).

**General procedure for synthesis of Methyl 3, 5-dimethoxy-4-(2-(4-methoxy phenyl) ethynyl) benzoate (5, 5A and 5B):**- To a stirred solution of methyl 4-iodo-3, 5-dimethoxybenzoate (150.0 mg, 0.510 mmol) in triethyl amine (6 mL), PdCl2 $(PPh_3)_2$ (45.0 mg, 0.064 mmol, 3 mol-%), CuI (12.0 mg, 0.063 mmol, 3 mol-%) and p-methoy-phenyl acetylene (79.0 mg, 0.598 mmol) for compound **5**; 1-Ethylnyl 4-Fluorobenzene (71.0 mg, 0.591 mmol) for compound **5A** and methyl 4-ethynylbenzoate (95.0 mg, 0.593 mmol) for compound **5B** were added and after being heated at 60$^0$C for 5h, the reaction mixture was cooled to rt and then extracted thrice with (50 mL) EtOAc. The combined EtOAc was dried over $Na_2SO_4$ and concentrated on rotary evaporator. The crude residue so obtained was purified by column chromatography (4% EtOAc/hexane), which afforded a solid compound-**5**

**(**140mg, 92%); a yellowish solid compound-**5A (**130 mg, 85%) and a solid compound-**5B** (100 mg, 65%).

**General procedure for synthesis of methyl 3-iodo-4-methoxy-2-(4-methoxyphenyl) benzofuran-6-carboxylate (6, 6A and 6B):-** To a solution of alkyne-**5, 5A and 5B** (725 mg) in $CH_2Cl_2$ (60 mL) $I_2$ (1140 mg) was gradually added in $CH_2Cl_2$ (15 mL) and the mixture was stirred at rt for 22 h. The reaction mixture was then diluted with (30 mL) of DCM and stirring was continued further for 10 min at rt. The excess amount of $I_2$ was removed by washing with a saturated aqueous solution of $Na_2S_2O_3$. The organic layer was dried over $Na_2SO_4$ and solvent removed under vacuum. The crude product so obtained was purified by column chromatography, which afforded compound-**6** (720mg, 96%), **6A** (700mg, 93%) and **6B** (65%).

**Methyl4-methoxy-3-(3,4,5-trimethoxy) 2(4methoxyphenyl) benzofuran-6-carboxylate (8B) :-** To a solution of iodobenzofuran-6 (500 mg, 1.14 mmol) in DMF/water (4:1, 20.5 mL), 3,4,5-trimethoxy phenyl boronic acid (350 mg, 1.3 mmol), sodium bicarbonate (133.4 mg, 1.6 mL) and $PdCl_2(PPh_3)_2$ (40 mg, 0.057 mmol) were added. The solution was, then stirred for 10 min at room temperature and then heated at 80 $^0$C for 10h. After cooling the reaction mixture, it was extracted thrice with EtOAc (50 mL). The organic layer was dried over $Na_2SO_4$ and concentrated on rotary evaporator. The crude so obtained was purified by column chromatography (4% EtOAc/hexane), which afforded solid compound-**8B** (630 mg, 89%).

**4-methoxy-3-(3,4,5-trimethoxyphenyl)-(4methoxyphenyl) benzofuran-6-carboxylic acid (8A):-** To a solution of final compound-8A (300 mg, 0.63 mmol) in methanol/water (9:1, 27.3 mL) potassium hydroxide (100 mg, 1.785 mmol) was added and the solution was heated for 6h at 80 $^0$C. The methanol was removed on rotary evaporator and the crude mixture was extracted thrice with EtOAc. The combined organic layer was dried over $Na_2SO_4$ and solvent removed on rotary evaporator. The residue so obtained was purified by column chromatography (4% EtOAc/hexane), which afforded a solid compound **8A** (285 mg, 97%).

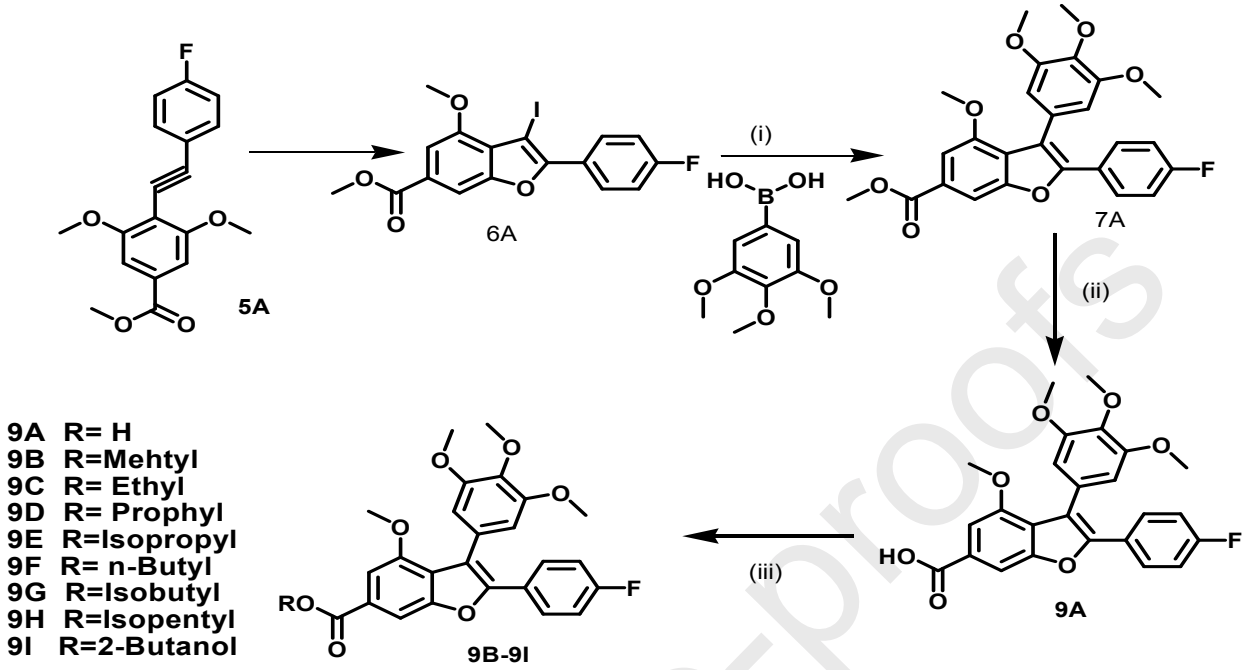
**Common method for the preparation of the substituted esters (8C-8G): -** To a solution of acid compound 8A (20 mg) in methanol (7 mL) was added 3drops of concentrated sulphuric acid and heated for 1h at 80 $^0$C. The methanol was removed on rotary evaporator and the crude residue was extracted thrice with EtOAc. The organic layer was dried over $Na_2SO_4$ and solvent removed on rotary evaporator. The crude was purified by column chromatography (4% EtOAc/hexanes), which afford the desired derivatives **(8C-8G;** 21- 23 mg, 90-98%).

**Scheme-2**

In scheme-2 compound **6A** and 3', 4', 5'-trimethoxy phenylboronic acid were coupled by Suzuki cross-coupling reaction to afford **7A**. Further **7A** was demethylated with 10% KOH in methanol and water (9:1) to afford final pharmacophore **9A**.

**Common method for the preparation of the substituted esters (9B-9I): -** To a solution of acid compound-**9A** (20 mg) in methanol (7 mL) 3drops of concentrated sulphuric acid was added. The solution was heated for 1h at 80 $^0$C. The methanol was removed under vacuum. The residue obtained was extracted thrice with (50 mL) EtOAc. The organic layer was dried over $Na_2SO_4$ and the solvent reduced under vacuum. The crude so obtained was purified by

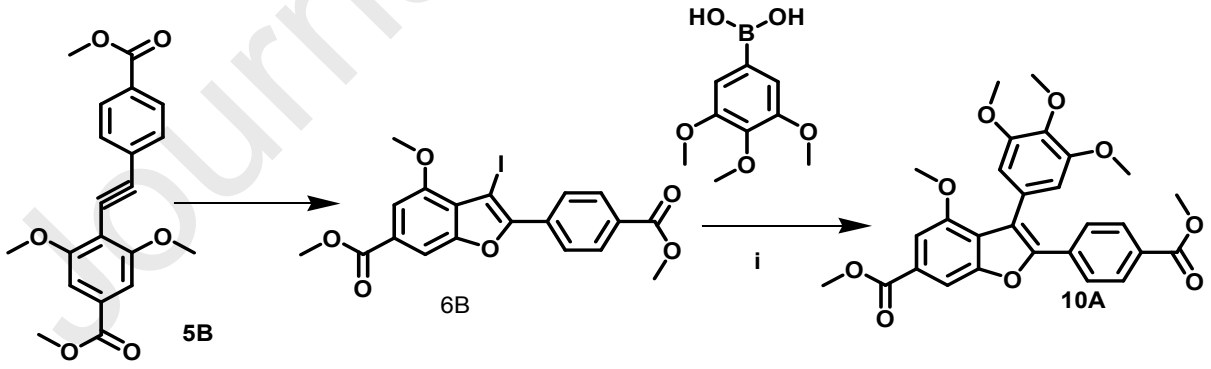
column chromatography (4% EtOAc/hexane), which afforded the desired products **9B-9I** (21-23mg; 90-98%).




**Scheme-2-**Reagent and condition: (i) Trimethoxy phenylboronic acid, NaHCO3, $(Ph_3P)_2PdCl_2$, DMF/$H_2O$ (4:1), 80 ˚C, 7h, 96%: (ii)KOH, methanol/ water (9:1), 80 ˚C, 6h, 98%. (iii) Conc-$H_2So_4$, methanol, temp 80°C, 4h, 98%.

**Scheme-3**

In scheme-3 the 3', 4', 5'- trimethoxy phenylboronic acid was hooked up to compound-**6B** by Suzuki cross-coupling reaction to afford the desired compound-**10A**.




**Scheme-3-Reagent and condition:** (i) Trimethoxy phenylboronic acid, NaHCO3, $(Ph_3P)_2PdCl_2$, DMF/$H_2O$ (4:1), 80 ˚C, 7h, 40%.

***In-vitro* anti-tubercular assay using *M. tuberculosis* (avirulent strain):**

**Determination of MIC:**

The Minimum Inhibitory Concentration (MICs) of 2,3-diaryl benzofuran derivatives and antitubercular drugs INH were determined against Mtb H37Ra by Agar dilution assay [**15**].

Briefly, 2,3-diaryl benzofuran derivatives were dissolved in dimethyl sulphoxide (DMSO) to make stock (5 mg/mL). Serial dilutions from stocks were also made in DMSO. To 1.9 mL MB 7H11 agar medium (in tubes, temp. 45-50ºC, with OADC supplement), 0.1 mL of a compound or DMSO (negative control) was added. The contents were mixed and allowed to solidify as slants. Three weeks old culture of Mtb H37Ra was harvested from L-J medium and its suspension (1 mg/mL equivalent to 108 bacilli approximately) was made in normal saline (containing 0.05% Tween-80). 10 µl of 1:10 dilution of this suspension (~105 bacilli) was inoculated into each tube and incubated at 37 ºC for 4 weeks. The lowest concentration of a compound up to which there was no visible growth of bacilli was its minimal inhibitory concentration (MIC).

### *In silico* studies

#### Identification of anti-Mtb targets, target preparation and molecular docking

The probable anti-Mtb targets were searched through STITCH database v5.0 (http://stitch.embl.de) and from reported literatures. Stitch database develop score-based interaction network between standard known compound and protein targets. The 3D crystallographic structures of identified targets *viz.,* polyketide synthase 13 (PDB: 5V3X), catalase peroxidase (PDB: 1SJ2), dihydrofolate reductase (PDB: 4M2X) and enoyl-ACP reductase (PDB: 4TRO) were retrieved through protein databank (http://www.pdb.org). The protein and ligand structures were prepared by using Autodock Vina (MGL, La Jolla, USA). Initially Autodock Vina prepared extended PDBQT file for receptor and ligands. The method included addition of polar hydrogens, atomic partial charges and AD4 atom type conversion, providing information about torsional degree of freedom. Afterward the receptor was implanted in 3D grid system with prob atoms at each grid point. The electrostatic and desolvation potentials of each atom type (C, O, N & H) with every grid point was calculated. Further, ligand conformation energy was calculated through grids. A docking protocol validation was performed by redocking study of crystallised ligand TAM1. The RMSD value for redocked and co-crystallised ligand was calculated by using Molsoft-chemist v3.8-6a 2018 software. A low RMSD value of 1.47 standardised the docking protocol. The receptor-ligand interaction results were analyzed through Discovery Studio visualizer.

#### Computational assessment for oral bioavailability and potential toxicity

The two major reasons for the new drug candidate's failure are the poor bioavailability and high toxicity. Hence, the oral bioavailability of the studied compounds were assessed through Lipinski's rule of five (RO5). While the toxicity parameters of the studied derivatives were assessed though the standard computational assessment tool TOPKAT (DS v3.5). TOPKAT utilizes its patented optimal predictive space (OPS) to calculate different toxicity parameters for query set compounds (Enslein, 1988). The oral bioavailability, blood-brain-barrier (BBB) penetration capacity and gastrointestinal (GI) absorption were assessed though DS ADME plot. This included the calculated parameters such as, plasma protein binding, CYP2D6 metabolism, hepatotoxicity, mouse and rat male/female carcinogenicity, developmental toxicity (DT), skin and ocular irritancy etc.

## RESULTS & DISCUSSION

As part of our drug discovery program, the starting material 3', 5'- dihydroxybenzoic acid was converted to pharmacophore **8** as given in scheme 1. Later on, using this pharmacophore,

seven derivatives (**8A** to **8G**) were prepared. Similarly, using 3', 4', 5'-Trimethoxy phenylboronic acid (**6A**) as starting material pharmacophore **9** was prepared as given in scheme 2 and using this Pharmacophore-**9** nine derivatives (**9A** to **9I**) were prepared. Compound-**10A** was prepared by hooking up 3', 4', 5'-trimethoxy phenylboronic acid to compound **6B** by Suzuki cross-coupling reaction as given in scheme 3. It was also observed that in Suzuki cross-coupling carbon-carbon bond formation reaction, the aryl groups containing electron-donating groups resulted in higher yields while the electron-withdrawing groups gave lower yields. All the compounds were characterized with the use of 1H, $^{13}C$ NMR and Mass spectroscopic data. Further, all the derivatives were evaluated for their antitubercular activity against the H37Ra avirulent strain (ATCC 25177) using isoniazid as the standard drug and the results are presented in (Table 1).

**Table 1:** MIC of 2, 3-diaryl benzofuran hybrids derivatives

| S.No | Code | MIC (ug/mL) |
|---|---|---|
| 1. | 8A | Not Active |
| 2. | 8B | Not Active |
| 3. | 8C | **50 ug/mL** |
| 4. | 8D | Not Active |
| 5. | 8E | Not Active |
| 6. | 8F | Not Active |
| 7. | 8G | Not Active |
| 8. | 9A | **50 ug/mL** |
| 9. | 9B | Not Active |
| 10. | 9C | Not Active |
| 11. | 9D | Not Active |
| 12. | 9E | **12.5 ug/mL** |
| 13. | 9F | Not Active |
| 14. | 9G | **50 ug/mL** |
| 15. | 9H | Not Active |
| 16. | 9I | Not Active |
| 17. | 10A | Not Active |
| Std Drug INH | | (0.025) **ug/mL** |
| No drug control | | ++++++ |

From the Table-1, it is evident that several derivatives were active in the range of 12.5 to 50µg/mL. Among all, derivative **9E** was the most active (MIC 12.5 µg/mL) followed by the derivatives **8C, 9A** and **9G** (MIC 50 µg/mL) while the starting material **7** (8B) was inactive. Further, Structure Activity Relationship (SAR) for these derivatives against the $H_{37}Ra$ avirulent strain can be derived. From the Table-1, it is evident that with the free acid group (**8A**) was inactive, but on increasing the ester chain length from methyl to ethyl, propyl, *iso*-propyl, *n*-butyl and *iso*-pentyl (**8B to 8G**) the activity dramatically increased in ethyl ester (**8C**) which may be due to increase in ester chain length, but after that increase in chain length resulted in loss of activity. The reason may be that the electron-donating aliphatic chains should be of medium size, which could fit well at the receptor site. Similarly, when we compare between the structures of **8A** and **9A,** there is only difference of $–OCH_3$ and –F groups at the C-4 (*p*) position of phenyl ring, but there was significant difference in their activity. The derivative **8A** was inactive while **9A** was active (MIC 50 µg/mL). Further, when other methyl, ethyl, propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *iso*-pentyl and 2-butanyl (**9B** to **9I**) ester derivatives of **9A** were evaluated for their antitubercular potential, it was found that isopropyl ester derivative (**9E**) was most active (MIC 12.5 µg/mL) followed by *iso*-butyl ester (MIC 50 µg/mL) derivative (**9G**), which is the same to that of starting material **9A** (MIC 50 µg/mL). This significant increase in **9E** activity may be further correlated to the increased electron-donating power of branched aliphatic chains of medium size, which could fit well at the receptor site. The last (4-methoxycarbonyl) phenyl benzofuran derivative (**10A**) was found inactive. From the above it may be concluded that (4-fluoro) phenyl group with medium size branched aliphatic chains played an important role in enhancing the antitubercular activity.

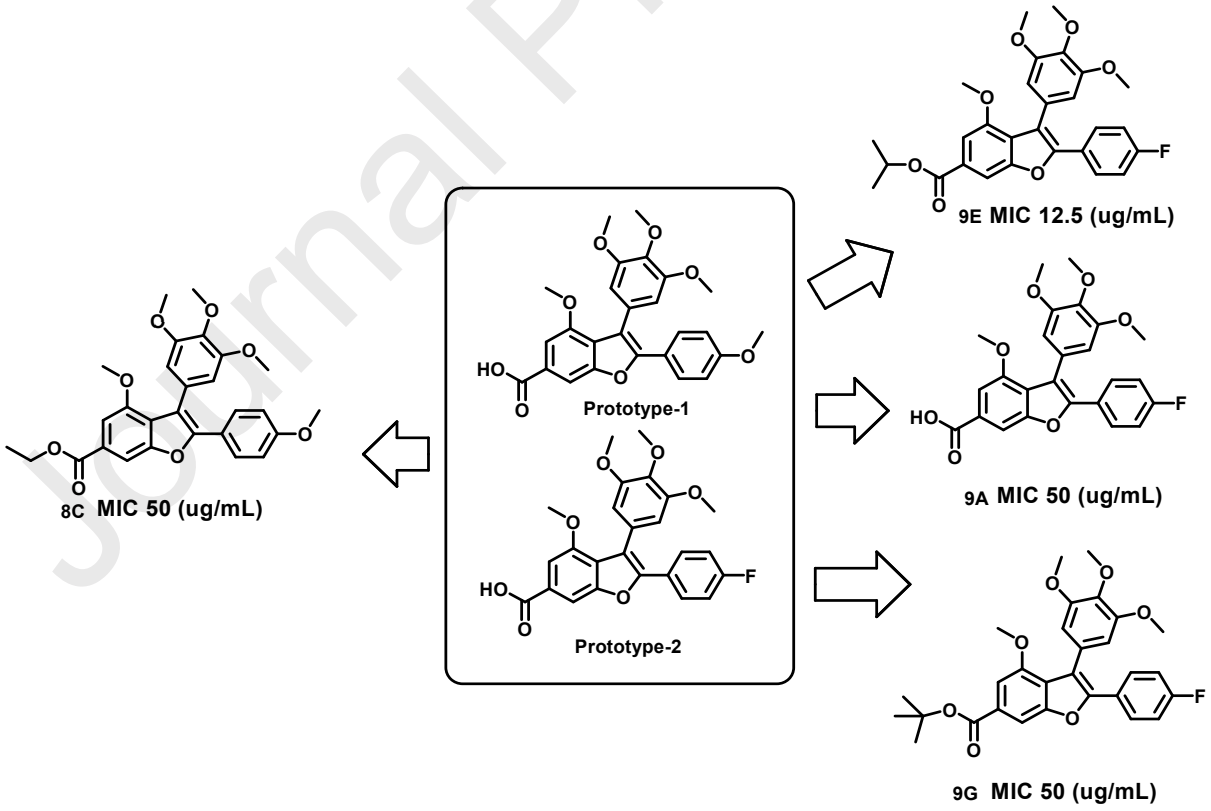



**Figure 4**: Best molecules of this series

**Molecular docking and mode of action study of derivatives on identified Mtb targets:**

Mycolic acid is one of the most essential components for Mtb virulence and drug resistance. Till now more than 20 enzymes have been discovered which are involved in Mtb mycolic acid synthesis. These are long chained fatty acids found abundantly in Mtb cell walls which provide high resistance to the bacteria against several antibiotics. Polyketide synthase 13 (PK13) is one of the essential enzymes involved in advance phase of mycolic acid synthesis in Mtb. PK13 exhibit five catalytic domains *viz*., one acyl transferase domain, two acyl carrier domains, one β-keto acyl-synthase domain and the C-terminal thioester (TE) domain that collectively contribute to the mycolic acid synthesis process. TM16 is a recently discovered benzofuran class based highly potent PK13 inhibitor. TM16 binds at TE domain of PK13 and block the availability of its catalytic centre for substrate binding. Here the synthesised active derivatives *viz*., **8C**, **9A**, **9E** and **9G** belong to the benzofuran class therefore we performed a molecular docking study against PK13. Based on STITCH database three additional putative targets namely catalase-peroxidase, dihydrofolate reductase and enoyl-ACP reductase were prioritized for ligand-target binding studies (Figure 6).

The results of docking binding affinity of studied derivatives are compiled in (Table 2 and Table 4).

**Table 2**: Molecular docking results of derivatives 8C, 9A, 9E, 9G against Mtb target PK13

| Name | Docking binding energy (kcal/mol) | PK13 (PDB: 5V3X) binding pocket residues within 4Å radius | Key amino acid & H-bond length (Å) |
|---|---|---|---|
| **ISONIAZID (positive control)** | -6.2 | SER-1533, LEU-1534, VAL-1562, ASN-1640, ILE-1643, ASP-1644, TYR-1663, ALA-1667, TYR-1674, HIS-1699 | TYR-1663 (2.90) |
| **TM16 (PK13 co-crystallized ligand)** | -9.6 | TYR-1582, GLN-1633, SER-1636, TYR-1637, ASN-1640, ARG-1642, ILE-1643, ASP-1644, TYR-1663, HIS-1664, ASP-1666, ALA-1667, PHE-1670, GLU-1671, TYR-1674 | ASP-1644 (2.55) |
| **8C** | -8.3 | ALA-1477, TRP-1532, SER-1533, TYR-1582, ASN-1640, ILE-1643, ASP-1644, TYR-1663, HIS-1664, ASP-1666, ALA-1667, PHE-1670, GLU-1671, HIS-1699, ILE-1700 | SER-1533 (3.07) |
| **9A** | -8.6 | ALA-1477, TRP-1532, SER-1533, ASN-1640, ILE-1643, ASP-1644, TYR-1663, HIS-1664, PHE-1670, GLU-1671, HIS-1699, ILE-1700 | SER-1533 (3.06) |
| **9E** | -8.7 | ALA-1477, TRP-1579, TYR-1582, VAL-1611, GLN-1633, TYR-1637, ASN-1640, HIS-1664, ASP-1666, ALA-1667, PHE-1670, GLU-1671, TYR-1674, ILE-1700 | GLN-1633 (2.92) |
| **9G** | -8.8 | ALA-1477, TRP-1579, TYR-1582, GLN-1633, TYR-1637, ASN-1640, HIS-1664, ASP-1666, ALA-1667, PHE-1670, GLU-1671, TYR-1674, ILE-1700 | GLN-1633 (3.03) |

Analysis of docking results against target PK13 indicate, the studied derivatives *viz*., **8C**, **9A**, **9E** and **9G** showed good binding affinity in comparison to control drug isoniazid. Derivatives

**9E** and **9G** showed better binding affinity of -8.7 kcal/mol and -8.8 kcal/mol than derivative **8C** and **9A** with

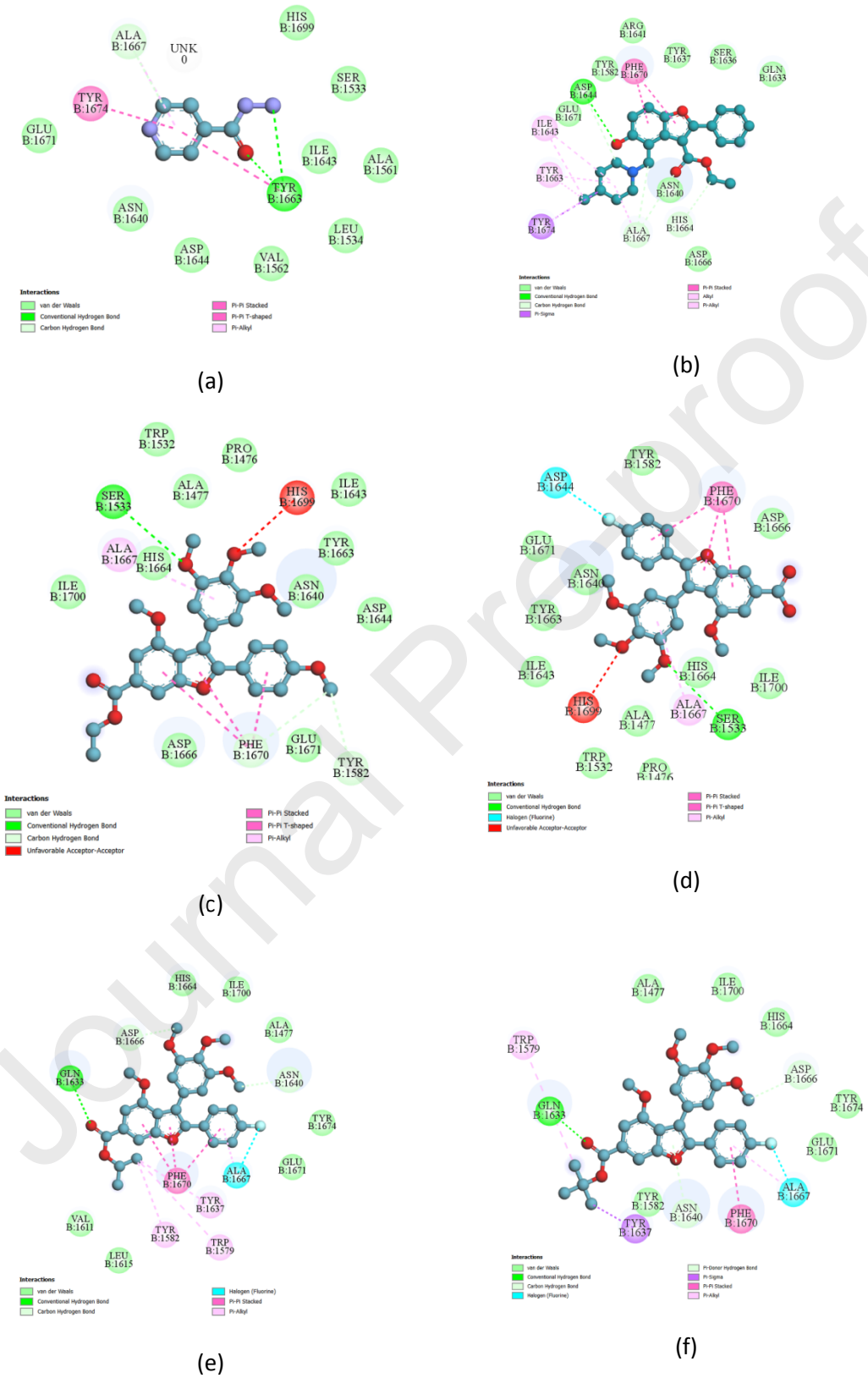



**Figure 5**: A 2D representation of binding mode of positive control Mtb drug Isoniazid and PK13 co-crystallized benzofuran based inhibitor named 'TM16' and synthesized novel

derivatives **8C, 9A, 9E** and **9G**. (a) propose binding pose of Isoniazid on PK13 binding site with docking binding energy -6.2 kcal/mol, (b) re-docking study of co-crystallized ligand TM16 on PK13 TE domain (benzofuran based inhibitor), (c) binding pose of derivative 8C on PK13 binding site with docking binding energy -8.3 kcal/mol, (d) binding pose of derivative 9A on PK13 binding site with docking binding energy -8.6 kcal/mol, (e) binding pose of derivative 9E on PK13 binding site with docking binding energy -8.7 kcal/mol and (f) binding pose of derivative 9G on PK13 binding site with docking binding energy -8.8 kcal/mol.

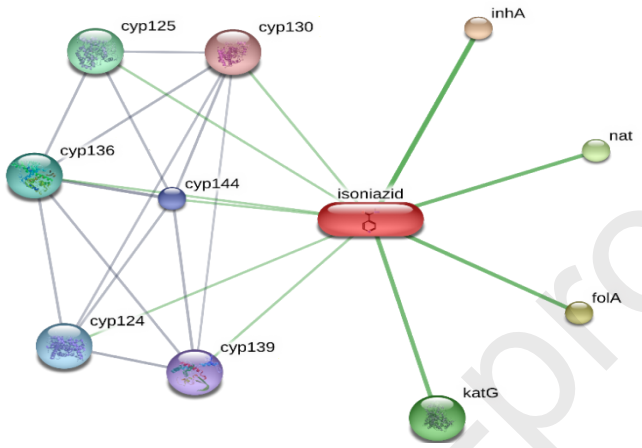



**Figure 6**: Mtb target prediction for positive control isoniazid through web-based tool STITCH v5.0 (http://stitch.embl.de). inhA, nat, folA and katG stands for enoyl-ACP reductase, arylamine V-acetyltransferase, dihydrofolate reductase and catalase peroxidase respectively.

**Table 3**: TOPKAT predicted toxicity and carcinogenic potency of positive control INZ (ISONIAZID) and synthesized derivatives, **8C**, **9A**, **9E** and **9G**

| **Name** | **Mouse Female** | **Mouse Male** | **Rat Female** | **Rat Male** | **Ames mutagenicity** | **Hepatotoxicity** | **DTP** |
|---|---|---|---|---|---|---|---|
| **INH** | Multi Carcinogen | Multi-Carcinogen | Multi-Carcinogen | Non-Carcinogen | Mutagen | True | Toxic |
| **8C** | Non-Carcinogen | Multi-Carcinogen | Single-Carcinogen | Single-Carcinogen | Non-Mutagen | True | Toxic |
| **9A** | Non-Carcinogen | Non-Carcinogen | Non-Carcinogen | Single-Carcinogen | Non-Mutagen | True | Toxic |
| **9E** | Non-Carcinogen | Single-Carcinogen | Non-Carcinogen | Single-Carcinogen | Non-Mutagen | True | Toxic |
| **9G** | Non-Carcinogen | Non-Carcinogen | Non-Carcinogen | Single-Carcinogen | Non-Mutagen | True | Toxic |

DTP; developmental toxicity

**Table 4 :** Compliance of docking binding energy results of derivatives **8C**, **9A**, **9E** and **9G** on putative Mtb targets catalase peroxidase, dihydrofolate reductase and enoyl-ACP reductase.

| Name | Catalase-peroxidase (PDB: 1SJ2) | Dihydrofolate reductase (PDB: 4M2X) | Enoyl ACP reductase (PDB: 4TRO) |
|---|---|---|---|
| **ISONIAZID (positive control)** | -6.0 | -5.6 | -5.9 |
| **8C** | -7.8 | -8.5 | -8.0 |
| **9A** | -8.5 | -8.5 | -8.6 |
| **9E** | -9.7 | -8.5 | -8.8 |
| **9G** | -8.9 | -9.0 | -8.9 |

binding energy of -8.3 kcal/mol and -8.6 kcal/mol respectively. However, the redocking study of TM16 the known PK13 inhibitor displayed highest binding affinity of -9.6 kcal/mol (Table 2). Further, the ligand-receptor interaction analysis revealed all derivatives bind well at inhibitor binding site of PK13. The interaction results displayed in (Table 2) indicated that the derivatives **8C** and **9A** form hydrogen bond with amino acid residue SER-1533 and trimethoxy phenyl fragment of the derivatives.

However they also indicated an unfavourable acceptor-acceptor hindrance with PK13 binding site amino acid residue HIS-1699 and trimethoxy phenyl moiety of **8C** and **9A** which is highlighted with red colour in Figure 5. On the other hand derivatives **9E** and **9G** are making hydrogen bond with carbonyl oxygen present at benzofuran moiety and amino acid residue GLN-1633. While, no unfavourable interaction was observed in the case of **9E** and **9G** derivatives. All derivatives also showed number of non-covalent interactions such as π-π T-shaped, π-π stacked, π-σ, π-alkyl and halogen interaction with aromatic rings of compounds (Figure 5). Here, figure 5 illustrate aromatic rings of benzofuran moiety of derivatives **8C** and **9A** make π-π T-shaped interaction with aromatic ring of amino acid residue PHE1670. Whereas derivatives **9E** and **9G** are making π-π stacked based interaction with amino acid residue PHE-1670. PK13 amino acid residue PHE1670 is located near to the acyl binding pocket and play critical role in TM16 binding. Derivatives **9E** and **9G** make hydrogen bond (green dotted line) of length 2.9 Å and 3.0 Å with amino acid residue GLN-1633. They also showed halogen-based interaction with fluoride group and binding site amino acid residue ALA-1667. Also, the results of docking binding affinity of derivatives **8C, 9A, 9E** and **9G** against other predicted targets viz., KatG, FolA and inhA indicated that the derivatives possess good binding affinity in comparison to FDA approved drug isoniazid (Table-4). Analysis of ligand-target interactions showed the derivatives make number of interactions with hydrophobic binding sites of targets KatG, FolA & inhA.

**Pharmacokinetic and toxicity assessment results**

The toxicity predictions results related to male/female mouse and rat, ames mutagenicity, indicate active derivative **9E** is non-carcinogenic and non-mutagenic in nature. However, other derivatives *viz*., **8C, 9G** and **9A** may show hepatotoxicity and developmental toxicity when prolong used. All studied derivatives showed less oral lethality in rat ($LD_{50}$) compared to drug isoniazid. A good plasma protein binding capacity of studied derivatives display high

biological half-life that influence the biological efficacy of derivatives. All studied derivatives were predicted to be CYP2D6 non-inhibitor which indicate the compounds are safe for drug-drug based interactions. Derivatives **9E** and **9G** were predicted to be safe for skin and ocular irritancy and sensitization parameters. All derivatives are aerobically degradable means they are non-persistent in nature (Table 3 and 4). Results of Lipinski's rule of five showed all the studied derivatives violate single rule of RO5 by crossing the cut-off limit for LogP value *i.e*., 5. Compliance of predicted toxicity parameters and RO5 are given in table (5).

**Table 5**: Parameter calculation for Lipinki's rule of five (RO5) violations

| Name | ALogP98 | PSA_2D (Å$^2$) | Molecular weight | No. of H-Acceptor | No. of Donor | No. of violation |
|---|---|---|---|---|---|---|
| INH | -0.423 | 67.912 | 137.139 | 2 | 2 | 0 |
| 8C | 5.584 | 83.435 | 492.517 | 7 | 0 | 1 |
| 9A | 5.231 | 86.39 | 452.428 | 6 | 1 | 1 |
| 9E | 6.183 | 74.505 | 494.508 | 6 | 0 | 1 |
| 9G | 6.388 | 74.505 | 508.535 | 6 | 0 | 2 |

**Conclusion:**

In summary, 2, 3-diaryl benzofuran derivatives were precisely designed and synthesized by the concise method. All the seventeen novel benzofuran synthesized derivatives were screed for their antitubercular activity against the H37Ra avirulent strain (ATCC 25177) using isoniazid as the standard drug. Among the seventeen derivatives, four showed antitubercular activity of which one (**9E**) was significantly active (MIC 12.5 ug/mL). From this study it may be concluded that (4-fluoro) phenyl group with medium size branched aliphatic chains played an important role in enhancing the antitubercular activity in benzofuran. Further, molecular docking results revealed that **9E** binds well at inhibitor binding site of Mtb drug target polyketide synthase 13 with binding energy of -8.7 kcal/mol. Amino acid residues GLN-1633 and PHE-1670 playing major role in hydrogen bonding and non-covalent based interactions with derivative **9E**. The computational oral bioavailability and toxicity assessment indicated that the derivative **9E** may show good pharmacokinetic properties and less toxicity. However, further studies are needed for QSAR guided lead optimization. These results may be of great help in anti-tubercular drug development from a very common, inexpensive synthon.

**CONFLICT OF INTEREST**

The authors confirm that they have no conflict of interest to declare.

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## References


[1] W.H.O. Global tuberculosis report, **2018**. https://www.who.int/tb/publications/global_report/gtbr2018_main_text_28Feb2019.pdf

[2] W.H.O.Global tuberculosis report, **2016**. https://apps.who.int/medicinedocs/en/d/Js23098en/

[3] Newman, D.J. Cragg, G.M. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod*. **2012**, 75, 311–335. DOI: 10.1021/np200906s

[4] Kingston, D.G. Modern natural products drug discovery and its relevance to biodiversity conservation. *J. Nat. Prod.* **2011**, 74, 496–511. DOI: 10.1021/np100550t

[5] Ashish Radadiya, AnamikShah, Bioactive benzofuran derivatives: An insight on lead developments, radioligands and advances of the last decade. Eur. J. Med. Chem. **2015**, 97, 356-376. https://doi.org/10.1016/j.ejmech.2015.01.021

[6] ZhiXu, ShijiaZhao, Zaoshengl, LianshunFeng, YinlingWang, FengZhang, Liuyangbai and JialunDeng, Benzofuran derivatives and their anti-tubercular, anti-bacterial activities. *Eur. J. Med. Chem.* **2019**, 162, 266-276. https://doi.org/10.1016/j.ejmech.2018.11.025

[7] Feng Gao, huaYang, TianyuLu, ZijianChen, LongMa, ZhiXu, PaulSchaffer, GuangmingLu, Design, synthesis and anti-mycobacterium activity evaluation of benzofuran-isatin hybrids. *Eur. J. Med. Chem.* **2018**, 159, 277-281. https://doi.org/10.1016/j.ejmech.2018.09.049

[8] Salomon, C. E. Schmidt, L. E. Natural Products as Leads for Tuberculosis Drug Development, *Curr. Top. Med. Chem.,* **2012**, *12*, 735-765. DOI: 10.2174/156802612799984526

[9] Komal Kalani, Vinita Chaturvedi, Sarfaraz Alam, Feroz Khan, and S. K. Srivastava, Anti-Tubercular Agents from *Glycyrrhiza glabra. Current Topics in Medicinal Chemistry, **2015**, 15, 1043-1049. DOI:* 10.2174/1568026615666150317223323

[10] Komal Kalani, Vinita Chaturvedi, Priyanka Trivedi, Sudeep Tondon and S.K. Srivastava, Dihydroartemisinin and its Analogs: A New Class of Antitubercular Agents *Current Topics in Medicinal Chemistry,* **2019***, 19,* 594-599. DOI: 10.2174/1568026619666190304142802

[11] Gaurav R. Dwivedi, Shikha Gupta, Sudeep Roy, Komal Kalani, Anirban Pal, Jay P. Thakur,Dharmendra Saikia, Ashok Sharma, Nandan S.Darmwal, Mahendra P. Darokar and S.K. Srivastava. Tricyclic Sesquiterpenes From Vetiveria zizanoides (L.) Nash as Antimycobacterial Agents *Chem Biol Drug Des.* **2013**, 82, 587–594. DOI: 10.1111/cbdd.12188

[12] Shikha Gupta , Gaurav R. Dwivedi , Mahendra P. Darokar , S.K. Srivastava, Antimycobacterial activity of fractions and isolated compounds from Vetiveria zizanioides. *Med Chem Res* **2012**, 21, 1283–1289 DOI: 10.1007/s00044-011-9639-8

[13] Harish C. Upadhyay, Jay Prakash Thakur , Dharmendra Saikia, S.K. Srivastava Anti-tubercular agents from Ammannia baccifera (Linn.) *Med Chem Res* **2013**, 22, 16–21 DOI: 10.1007/s00044-012-9998-9

[14] S. Singh, J. K. Kumar, D. Saikia, K. Shanker, J. P. Thakur, A. S. Negi, Suchitra Banerjee. A bioactive labdane diterpenoid from *Curcuma amada* and its semi synthetic analogues as antitubercular agents, *Eur. J. Med. Chem.* **2010**, 45, 4379-4382. https://doi.org/10.1016/j.ejmech.2010.06.006

[15] J. Kenneth McClatchy, Susceptibility Testing of Mycobacteria. *Laboratory Medicine* **1978**, *9*, 47-52. DOI: https://doi.org/10.1093/labmed/9.3.47

**Supplementary file**

## NMR Spectroscopy and Mass spectrometry data

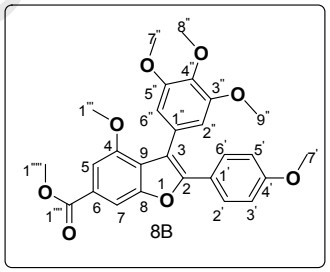
### Spectroscopic data:

**Methyl 4-iodo-3, 5-dimethoxy benzoate (4)**: $^{1}$H NMR (500MHz, $CDCl_3$): δ 7.09 (s, 2H, H-2 & 6), 3.87 (s, 3H, $OCH_3$, H-8), 3.5 (s, 6H, $2OCH_3$, H-9 & 10 ).$^{13}$C NMR ($CDCl_3$, 125MHz) ppm 131.48 (C, C-1), 104.36 (CH, C-2&6), 84.60 (C, C-4), 159.18 (C, C-3&5), 165.85 (CO, C-7), 52.51 ($CH_3$, C-8), 56.73 (OCH3, C-9&10).

**Methyl 3, 5-dimethoxy-4-(2-(4-methoxy phenyl) ethynyl) benzoate (5):** $^{1}$H NMR (500MHz, $CDCl_3$): δ 7.35 (s, 2H, H-2 & 6), 3.81 (s, 3H, $OCH_3$, H-8), 3.79 (s, 9H, $3OCH_3$, H-9, 10 & 7") 7.36 (d, 2H,H-2" & 6"), 7.84 (d, 2H, H-3" & 5"). $^{13}$C NMR ($CDCl_3$, 125MHz) ppm 131.79 (C, C-1), 104.68 (CH, C-2 & 6), 159.40 (C, C-3 & 5), 84.60 (C, C-4), 166.50 (CO, C-7). 52.26 ($CH_3$, C-8), 55.17 (OCH3, C-9 & 10), 93.00 (C, C-1' & 2' ), 115.00 (C, C-1"), 133.09 (CH, C-2" & 6"), 113.78 (C, C-3" & 5" ), 162.60 (C, C-4" ), 56.17 ($OCH_3$, C-7").

**Methyl 3-iodo-4-methoxy-2-(4-methoxyphenyl) benzofuran-6-carboxylate (6):** $^{1}$H NMR (500MHz, $CDCl_3$): δ 7.37 (s, 1H, H-5), 7.83 (s, 1H, H-7), 8.09 (d, 2H, H-2' & 6'), 7.02 (d, 2H, H-3' & 5'), 3.86 (s, 3H, $OCH_3$, H-7'), 3.94 (s, 3H, $OCH_3$, H-1"), 4.00 (s, 3H, $OCH_3$, H-1""). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 155.38 (C, C-2), 55.82 (C, C-3), 153.36 (C, C-4), 106.36 (CH, C-5), 126.96 (C, C-6), 105.05 (CH, C-7), 160.57 (C, C-8), 122.25 (C, C-9), 124.16 (C, C-1'), 129.63 (CH, C-2' & 6'), 113.88 (CH, C-3' & 5'), 162.70 (C, C-4'), 53.24 ($CH_3$, C-7'), 55.41 ($CH_3$, C-1"), 167.03 (CO, C-1""), 52.35 ($CH_3$, C-1"").

**Methyl 4-methoxy-3-(3,4,5-trimethoxy)-2-(4methoxyphenyl) benzofuran-6-carboxylate (7** and **8B) :**




Solid with mp 149.9 $^{0}$C. $^{1}$H NMR (500MHz, $CDCl_3$): δ 7.37 (s, 1H, H-5), 7.83 (s, 1H, H-7), 8.08 ( d, 2H, H-2' & 6' J= 9.0 Hz), 6.97 (d, 2H, H-3' and 5' J= 8.5 Hz), 6.56 (s, 1H, H-2" & 6'') 6.58 (s, 1H, H-6'') 3.84 (s, 9H, $OCH_3$, H-7',7'' & 9"), 3.94 ($OCH_3$, 3H , H-8"), 3.94 (s, 3H, $OCH_3$, H-1'"), 3.99 (s, 3H, $OCH_3$, H-1""). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 154.41 (C, C-2), 60.85 (C, C-3), 153.36 (C, C-4), 106.36 (CH, C-5), 126.97 (C, C-6), 105.05 (CH, C-7), 155.37 (C, C-8), 122.24 (C, C-9), 124.15 (C, C-1'), 129.62 (CH, C-2' & 6'), 113.88 (CH, C-3' & 5'), 160.58 (C, C-4'), 53.23 ($CH_3$, C-7'), 138.09 (C,C-1")'.105.19 (C, C-2"& 6"), 153.53 (C,

C-3'' &5''), 139.30 (C, C-4''), 55.81 ($OCH_3$, C-7'' & 9''), 56.07 ($OCH_3$, C-8''), 55.40 ($CH_3$, C-1'''), 167.01 (CO, C-1''''), 52.34 ($OCH_3$, C-1'''''') ESI-MS (MeOH): For $C_{27}H_{26}O_8$, 517 $[M+K]^+$.

**4-methoxy-3-(3,4,5-trimethoxyphenyl)-2-(4methoxyphenyl) benzofuran-6-carboxylic acid (8A):** Solid with mp 242.5 $^0$C . $^1$H NMR (500MHz, $CDCl_3$): δ 7.37 (s, 1H, H-5), 8.15 (s, 1H, H-7), 8.00 (d, 2H, H-2' & 6' J= 6.5 Hz), 7.04 ( d, 2H, H-3' & 5' J= 6.5 Hz ), 6.57 (s, 1H, H-2'' ) 6.59 (s, 1H, H-6'') , 3.83 (s, 9H, $OCH_3$, H-7' , 7'' & 9''), 3.94 ($OCH_3$, 3H , H-8''), 3.75 (s, 3H, $OCH_3$, H-1'''), ppm: $^{13}$C NMR ($CDCl_3$, 125 MHz): δ 152.53 (C, C-2), 119.97 (C, C-3), 151.07 (C, C-4), 103.77 (CH, C-5), 127.44 (C, C-6), 103.32 (CH, C-7), 152.20 (C, C-8), 112.11 (C, C-9), 121.79 (C, C-1'), 129.68 (CH, C-2' & 6'), 112.58 (CH, C-3' & 5'), 151.07 (C, C-4'), 58.20 ($CH_3$, C-7'), 129.91 (C,C-1''), 103.77 (C, C-2''& 6''), 152.20 (C, C-3'' & 5'' ), 129.91 (C, C-4''), 53.46 ($OCH_3$, C-7'' & 9''), 53.94 ($OCH_3$, C-8''), 53.46 ($CH_3$, C-1'''), 158.42 (CO, C-1'''') ppm. ESI-MS (MeOH): for $C_{26}H_{24}O_8$, 465 $[M+H]^+$.

**Ethyl 4-methoxy-3-(3, 4, 5-trimethoxyphenyl)-2-(4-methoxyphenyl) benzofuran-6-carboxylate (8C) :** white amorphous. In $^1$H NMR (500MHz, $CDCl_3$): δ 7.37 (s, 1H, H-5), 7.78 (s, 1H, H-7), 7.82 ( d, 2H, H-2' & 6' J= 8.5 Hz), 7.01 (d, 2H, H-3' and 5' J= 9.0 Hz), 6.57 (s, 1H, H-2'' ) 6.59 (s, 1H, H-6'') , 3.85 (s, 9H, $OCH_3$, H-7' , 7'' & 9''), 3.87 (s, 3H , $OCH_3$, H-8''), 4.00 (s, 3H, $OCH_3$, H-1'''), 4.40 (m, $CH_2$, 2H, H-1''''), 1.42 (t, 3H, $CH_3$, H-2'''' J= 6.5 Hz). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 154.41 (C, C-2), 61.26 (C, C-3), 153.35 (C, C-4), 106.30 (CH, C-5), 126.73 (C, C-6), 105.06 (CH, C-7), 154.97 (C, C-8), 122.82 (C, C-9), 124.20 (C, C-1'), 129.61 (CH, C-2' & 6'), 113.88 (CH, C-3' & 5'), 160.32 (C, C-4'), 53.23 ($CH_3$, C-7'), 139.30 (C,C-1'')'.105.22 (C, C-2''& 6''), 153.35 (C, C-3'' &5''), 139.30 (C, C-4''), 55.80 ($OCH_3$, C-7'' & 9''), 56.08 ($OCH_3$, C-8''), 55.40 ($CH_3$, C-1'''), 167.01 (CO, C-1''''), 61.09 ($CH_2$, C-1''''), 14.38 ($CH_3$, C-2''''). ESI-MS for $C_{28}H_{28}O_8$ (MeOH): 493 $[M+H]^+$

**Propyl 4-methoxy-3-(3,4,5-trimethoxyphenyl)-2-(4-methoxyphenyl)benzofuran-6-carboxylate (8D) :** White amorphous. In $^1$H NMR (500MHz, $CDCl_3$): δ 7.37 (s, 1H, H-5), 7.78 (s, 1H, H-7), 7.79 (d, 2H, H-2' & 6' J= 8.5 Hz), 6.99 (d, 2H, H-3' & 5' J= 7.0 Hz ), 6.96 (s, 1H, H-2'' ) 6.98 (s, 1H, H-6''), 3.85 (s, 9H, $OCH_3$, H-7',7'' & 9''), 4.00 ( s, 3H , $OCH_3$, H-8''), 3.85 (s, 3H, $OCH_3$, H-1'''), 4.31 (t, 2H, $CH_2$, H-1'''' J= 6.5 Hz) 1.82 (m, 2H, $CH_2$, H-2'''') 1.4 (t, 3H, $CH_3$, H-3'''' J= 7.0 Hz). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 154.41 (C, C-2), 66.67 (C, C-3), 153.83 (C, C-4), 106.49 (CH, C-5), 126.61 (C, C-6), 105.08 (CH, C-7), 154.97 (C, C-8), 122.81 (C, C-9), 124.20 (C, C-1'), 129.60 (CH, C-2' & 6'), 113.88 (CH, C-3' & 5'), 160.33 (C, C-4'), 52.35 ($CH_3$, C-7'), 139.30 (C,C-1'')'.105.24 (C, C-2''& 6''), 153.83 (C, C-3'' &5''), 139.30 (C, C-4''), 55.78 ($OCH_3$, C-7'' & 9''), 56.07 ($OCH_3$, C-8''), 55.39 ($CH_3$, C-1'''), 167.06 (CO, C-1''''), 66.67 ($CH_2$, C-1''''') 22.20 ($CH_2$, C-2''''') 10.58 ($CH_3$, C-3'''''). ESMS for $C_{29}H_{30}O_8$ (MeOH): 507 $[M+H]^+$

**Isopropyl 4-methoxy-3-(3, 4, 5-trimethoxyphenyl)-2-(4-methoxyphenyl) benzofuran-6-carboxylate (8E):** Viscous compound. In $^1$H NMR (500MHz, $CDCl_3$): δ 7.37 (s, 1H, H-5), 8.08 (s, 1H, H-7), 7.84 (d, 2H, H-2' & 6' J= 6.5 Hz), 7.01 (d, 2H, H-3' & 5' J= 9.0 Hz), 6.57 (s, 1H, H-2'' ) 6.59 (s, 1H, H-6''), 3.86 (s, 9H, $OCH_3$, H-7' , 7'' & 9''), 3.87 (s, 3H , $OCH_3$, H-8''), 3.87 (s, 3H, $OCH_3$, H-1'''), 5.27 (m, 1H, CH, H-1''''), 1.40 (d, 6H, 2X$CH_3$, H-2'''' & 3''''). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 154.41 (C, C-2), 115.90 (C, C-3), 153.32 (C, C-4), 106.24 (CH, C-5), 126.59 (C, C-6), 105.05 (CH, C-7), 155.24 (C, C-8), 122.32 (C, C-9), 124.07 (C, C-1'), 129.59 (CH, C-2' & 6'), 113.88 (CH, C-3' & 5'), 160.54 (C, C-4'), 55.40 ($CH_3$, C-7'), 132.23 (C,C-1'')'.105.19 (C, C-2''& 6''), 153.53 (C, C-3'' &5''), 139.31 (C, C-4''), 55.81 ($OCH_3$,

C-7'' & 9''), 56.07 ($OCH_3$, C-8''), 55.40 ($CH_3$, C-1'''), 166.46 (CO, C-1''''), 68.70 ($CH_2$, C-1''''), 22.71 ($CH_3$, C-2'''' & 3''''''). ESMS for $C_{29}H_{30}O_8$ (MeOH): 507 $[M+H]^+$

***n*-Butyl 4-methoxy-3-(3,4,5-trimethoxyphenyl)-2-(4-methoxyphenyl) benzofuran-6-carboxylate (8F):** Viscous compound. In $^1H$ NMR (500MHz, $CDCl_3$): δ 7.37 (s, 1H, H-5), 8.09 (s, 1H, H-7), 7.78 (d, 2H, H-2' & 6' J= 9.0 Hz), 7.35 (d, 2H, H-3'& 5' J= 9.5 Hz), 6.82 (s, 1H, H-2'' ) 6.96 (s, 1H, H-6''), 3.84 (s, 9H, $OCH_3$, H-7'' , 7'' & 9''), 3.99 ($OCH_3$, 3H , H-8''), 3.89 (s, 3H, $OCH_3$, H-1'''), 4.34 (t, 2H, $CH_2$, H-1'''' J= 6.5 Hz) 1.77 (m, 2H, $CH_2$, H-2'''') 1.50 (m, 2H, $CH_2$, H-3''''), 0.87 (t, 3H, $CH_3$, H-4'''' J= 7.0 Hz). $^{13}C$ NMR ($CDCl_3$, 125 MHz): ppm 154.40 (C, C-2), 115.11 (C, C-3), 153.53 (C, C-4), 106.47 (CH, C-5), 126.60 (C, C-6), 104.34 (CH, C-7), 154.96 (C, C-8), 122.80 (C, C-9), 124.17 (C, C-1'), 129.76 (CH, C-2' & 6'), 113.87 (CH, C-3' & 5'), 160.32 (C, C-4'), 52.34 ($CH_3$, C-7'), 132.07 (C,C-1'')'.106.24 (C, C-2''& 6''), 153.35 (C, C-3'' &5''), 139.29 (C, C-4''), 55.78 ($OCH_3$, C-7'' & 9''), 56.07 ($OCH_3$, C-8''), 55.39 ($CH_3$, C-1'''), 167.02 (CO, C-1''''), 66.11 ($CH_2$, C-1'''') 31.94 ($CH_2$, C-2'''') 19.35 ($CH_2$, C-3''''), 13.82 ($CH_3$, C-4'''') ppm. ESMS for $C_{30}H_{32}O_8$ (MeOH): 539 $[M+H+NH_4]^+$

**Isopentyl 4-methoxy3-(3, 4, 5-trimethoxyphenyl)-2-(4-methoxyphenyl) benzofuran-6-carboxylate (8G):** Viscous compound. In $^1H$ NMR (500MHz, $CDCl_3$): δ 7.37 (s, 1H, H-5), 8.10 (s, 1H, H-7), 7.79 (d, 2H, H-2' & 6' J= 8.5 Hz),, 7.00 (d, 2H, H-3'& 5' J= 8.5 Hz), 6.97 (s, 1H, H-2'' ) 6.99 (s, 1H, H-6''), 3.86 (s, 9H, $OCH_3$, H-7'' , 7'' & 9''), 3.88 (s, 3H , $OCH_3$, H-8''), 4.00 (s, 3H, $OCH_3$, H-1'''), 4.38 (t, 2H, $CH_2$, H-1'''' J= 6.0 Hz) 1.70 (m, 2H, $CH_2$, H-2''''), 1.82 (m, 1H, CH, H-3''''), 1.29 (s, 6H, $CH_3$, H-4'''' & 5''''). $^{13}C$ NMR ($CDCl_3$, 125 MHz): ppm 154.96 (C, C-2), 114.36 (C, C-3), 153.35 (C, C-4), 106.48 (CH, C-5), 126.61 (C, C-6), 104.34 (CH, C-7), 155.31 (C, C-8), 122.80 (C, C-9), 124.19 (C, C-1'), 129.60 (CH, C-2' & 6'), 113.88 (CH, C-3' & 5'), 160.32 (C, C-4'), 53.23 ($CH_3$, C-7'), 130.27 (C, C-1''), 106.24 (C, C-2''& 6''), 153.35 (C, C-3'' &5''), 139.31 (C, C-4''), 55.79 ($OCH_3$, C-7'' & 9''), 56.07 ($OCH_3$, C-8''), 55.40 ($CH_3$, C-1'''), 167.05 (CO, C-1''''), 63.79 ($CH_2$, C-1''''), 37.50 ($CH_2$, C-2''''), 25.39 ($CH_2$, C-3''''), 22.71 ($2CH_3$, C-4'''' & 5'''') ppm. ESMS for $C_{31}H_{34}O_8$ (MeOH): 552 $[M+H]^+$

**2-(4-fluorophenyl)-4-methoxy-3-(3,4,5-trimethoxyphenyl) benzofuran-6-carboxylicacid (9A)**: Half white solid with mp 253.0 $^0C$. In $^1H$ NMR (500MHz, $CDCl_3$): δ 7.71 (bs, 1H, H-7), 7.44 (bs, 1H, H-5), 7.44 ( d, 2H, H-2' & 6' J= 8.0 Hz), 7.45 (d, 2H, H-3'& 5' J= 8.0 Hz), 6.88 (s, 2H, H-2'' & 6'') , 3.83 (s, 9H, $OCH_3$, H-7'' , 8'' , 9'' ), 3.81 (s, 3H, $OCH_3$, H-1'''). ppm $^{13}C$ NMR ($CDCl_3$, 125 MHz): δ 139.28 (C, C-2), 119.32 (C, C-3), 151.83 (C, C-4), 104.23 (CH, C-5), 128.56 (C, C-6), 104.42 (CH, C-7), 161.08 (C, C-8), 123.50 (C, C-9), 125.52 (C, C-1'), 128.56 (CH, C-2' & 6'), 116.30 (CH, C-3' & 5'), 163.04 (C, C-4'), 132.24 (C, C-1''), 104.42 (C, C-2''& 6''), 160.68 (C, C-3''&5''), 133.05 (C, C-4''), 56.15 ($OCH_3$, C-7'', 8'', 9''&1'''), 167.00 (CO, C-1''''). ESI-MS (MeOH): for $C_{25}H_{21}FO_7$, 453 $[M+H]^+$.

**Methyl 2-(4-fluorophenyl)-4-methoxy-3-(3,4,5-trimethoxyphenyl) benzofuran-6-carboxylate( 9B)** : Viscous compound . In $^1H$ NMR (500MHz, $CDCl_3$): δ 7.38 (bs, 1H, H-5), 7.84 (bs, 1H, H-7), 8.13 (d, 2H, H-2' & 6' J= 9.0 Hz), 7.19 (d, 2H, H-3'& 5' J= 9.0 Hz), 7.16 (s, 2H, H-2'' & 6''), 3.93 (s, 12H, $OCH_3$, H- 7'' , 8'' , 9'' & 1'''), 4.00 (s, 3H , $OCH_3$, H-1''''). $^{13}C$ NMR ($CDCl_3$, 125 MHz): ppm 154.55 (C, C-2), 115.69 (C, C-3), 153.59 (C, C-4), 106.39 (CH, C-5), 127.57 (C, C-6), 105.15 (CH, C-7), 154.36 (C, C-8), 123.92 (C, C-9), 125.94 (C, C-1'), 128.55 (CH, C-2' & 6'), 115.51 (CH, C-3' & 5'), 162.31 (C, C-4'), 130.10 (C,C-1''), 105.15 (C, C-2''& 6''), 153.59 (C, C-3'' &5''), 132.33 (C, C-4''), 55.81 ($OCH_3$, C-7'' & 9''), 56.35 ($OCH_3$, C-8''), 54.61 ($CH_3$, C-1'''), 166.85 (CO, C-1''''), 52.34 ($OCH_3$, C-1''''). ESMS for $C_{26}H_{23}O_7$ (MeOH): 467 $[M+H]^+$

**Ethyl 2-(4-fluorophenyl)-4-methoxy-3-(3, 4, 5-trimethoxyphenyl) benzofuran-6-carboxylate (9C)**: **M.P**: 103.5 $^{0}$C .In $^{1}$H NMR (500MHz, $CDCl_3$): δ 7.58 (bs, 1H, H-5), 7.57 (bs, 1H, H-7), 7.56 (d, 2H, H-2' & 6' J= 8.5 Hz), 7.02 (d, 2H, H-3'& 5' J= 8.5 Hz), 7.16 (s, 2H, H-2'' & 6'') , 3.96 (s, 12H, $OCH_3$, H- 7'' , 8'' , 9'' & 1'''), 4.34 (m, 2H , $CH_2$, H-1''''), 1.40 (t, 3H , $CH_3$, H-2'''' J= 6.5 Hz), ppm: $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 154.55 (C, C-2), 115.69 (C, C-3), 153.59 (C, C-4), 106.39 (CH, C-5), 127.57 (C, C-6), 105.15 (CH, C-7), 154.36 (C, C-8), 123.92 (C, C-9), 125.94 (C, C-1'), 128.55 (CH, C-2' & 6'), 115.51 (CH, C-3' & 5'), 162.31 (C, C-4'), 130.10 (C,C-1''), 105.15 (C, C-2''& 6''), 153.59 (C, C-3'' &5''), 132.33 (C, C-4''), 55.81 ($OCH_3$, C-7'' & 9''), 56.35 ($OCH_3$, C-8''), 54.61 ($CH_3$, C-1'''), 166.09 (CO, C-1''''), 61.41 ($CH_2$, C-1''''), 14.10 ($CH_3$, C-2'''') ppm. ESI-MS (MeOH): for $C_{27}H_{25}FO_7$, 481 $[M+H]^+$

***n*-Propyl 2-(4-fluorophenyl)-4-methoxy-3-(3, 4, 5-trimethoxyphenyl) benzofuran-6-carboxylate (9D):** Yellowish viscous compound. In $^{1}$H NMR (500MHz, $CDCl_3$): δ 7.24 (s, 1H, H-5), 7.87 (s, 1H, H-7), 7.56 (d, 2H, H-2' & 6' J= 8.5 Hz), 7.14 (d, 2H, H-3'& 5' J= 7.5 Hz), 7.03 (s, 1H, H-2'' ) 7.04 (s, 1H, H-6''), 3.95 (s, 9H, $OCH_3$, H-7'' , 9'' & 1'''), 4.00 (s, 3H , $OCH_3$, H-8''), 4.30 (t, 2H, $CH_2$, H-1'''' J= 6.5 Hz) 1.79 (m, 2H, $CH_2$, H-2''''), 0.87 (t, 3H, $CH_3$, H-3'''' J= 7.0 Hz). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 151.95 (C, C-2), 119.59 (C, C-3), 152.81 (C, C-4), 106.00(CH, C-5), 126.92 (C, C-6), 104.41 (CH, C-7), 156.43 (C, C-8), 114.07 (C, C-9), 126.99 (C, C-1'), 126.99 (CH, C-2' & 6'), 116.13 (CH, C-3' & 5'), 163.58 (C, C-4'), 131.42 (C,C-1'')'.105.15 (C, C-2''& 6''), 156.43 (C, C-3'' &5''), 139.29 (C, C-4''), 56.03 ($OCH_3$, C-7'' , 8'' , 9''& 1'''), 166.15 (CO, C-1''''), 67.04 ($CH_2$, C-1''''), 22.70 ($CH_2$, C-2''''), 10.50 ($CH_3$, C-3''''). ESMS for $C_{28}H_{27}FO_7$ (MeOH): 495 $[M+H]^+$

**Isopropyl 2-(4-fluorophenyl)-4-methoxy-3-(3,4,5-trimethoxyphenyl) benzofuran-6-carboxylate (9E):** viscous compound. In $^{1}$H NMR (500MHz, $CDCl_3$): δ 7.23 (s, 1H, H-5), 7.58 (s, 1H, H-7), 7.56 (d, 2H, H-2' & 6' J= 9.0 Hz), 7.02 (d, 2H, H-3'& 5' J= 9.0 Hz), 6.59 (s, 1H, H-2'' ) 6.61 (s, 1H, H-6'') , 3.96 (s, 12H, $OCH_3$, H-7'', 8'', 9'' & 1'''), 5.27 (m, 1H, CH, H-1''''), 1.39 (s, $CH_3$, 6H, H-2'''' & 3''''). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 150.31 (C, C-2), 115.43 (C, C-3), 150.31 (C, C-4), 106.24 (CH, C-5), 126.59 (C, C-6), 105.05 (CH, C-7), 155.24 (C, C-8), 122.32 (C, C-9), 124.07 (C, C-1'), 129.59 (CH, C-2' & 6'), 113.88 (CH, C-3' & 5'), 160.54 (C, C-4'), 55.40 ($CH_3$, C-7'), 132.23 (C,C-1'')'.105.19 (C, C-2''& 6''), 153.53 (C, C-3'' &5''), 139.31 (C, C-4''), 55.81 ($OCH_3$, C-7'' & 9''), 56.07 ($OCH_3$, C-8''), 55.40 ($CH_3$, C-1'''), 166.46 (CO, C-1''''), 68.70 ($CH$, C-1''''), 22.71 ($CH_3$, C-2'''' & 3''''). ESMS for $C_{28}H_{27}FO_7$ (MeOH): 495 $[M+H]^+$

***n*-Butyl-2-(4-fluorophenyl)-4-methoxy-3-(3,4,5-trimethoxyphenyl) benzofuran- 6-carboxylate (9F):** Viscous compound. In $^{1}$H NMR (500MHz, $CDCl_3$): δ 7.24 (s, 1H, H-5), 7.58 (s, 1H, H-7), 7.56 (d, 2H, H-2' & 6' J= 9.0 Hz), 7.02 (d, 2H, H-3'& 5' J= 9.0 Hz), 6.94 (s, 1H, H-2'' ) 7.01 (s, 1H, H-6''), 3.95 (s, 9H, $OCH_3$, H-7'' , 9'' & 1'''), 4.00 (s, 3H , $OCH_3$, H-8''), 4.34 (t, 2H, $CH_2$, H-1'''' J= 6.5 Hz), 1.77 (m, 2H, $CH_2$, H-2''''), 1.46 (m, 2H, $CH_2$, H-3''''), 0.87 (t, 3H, $CH_3$, H-4'''' J= 7.0 Hz). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 142.77 (C, C-2), 119.60 (C, C-3), 151.96 (C, C-4), 106.53 (CH, C-5), 126.92 (C, C-6), 104.41 (CH, C-7), 156.43 (C, C-8), 114.07 (C, C-9), 126.99 (C, C-1'), 129.68 (CH, C-2' & 6'), 115.60 (CH, C-3' & 5'), 163.58 (C, C-4'), 130.62 (C,C-1'')'.105.16 (C, C-2''& 6''), 151.96 (C, C-3'' &5''), 139.28 (C, C-4''), 56.32 ($OCH_3$, C-7'' , 8'' , 9''& 1'''), 166.17 (CO, C-1''''), 65.35 ($CH_2$, C-1'''') 33.83 ($CH_2$, C-2'''') 22.70 ($CH_2$, C-3''''), 14.12 ($CH_3$, C-4''''). ESMS for $C_{29}H_{29}FO_7$ (MeOH): 509 $[M+H]^+$

***tert*-Butyl-2-(4-fluorophenyl)-4-methoxy-3-(3,4,5-trimethoxyphenyl)benzofuran-6-carboxylate (9G):** Yellowish solid with mp 159.5 $^{0}$C . In $^{1}$H NMR (500MHz, $CDCl_3$): δ 7.19

(s, 1H, H-5$_{)}$, 7.54 (bs, 1H, H-7), 7.52 (d, 2H, H-2$^{'}$ & 6$^{'}$ J= 8.5 Hz), 6.98 (d, 2H, H-3$^{'}$& 5$^{'}$ J= 8.5 Hz), 6.97 (s, 1H, H-2$^{''}$ ) 6.98 (s, 1H, H-6''), 3.91 (s, 12H, $OCH_3$, H-7'', 8$^{''}$, 9$^{''}$ & 1'''), 1.20 (s, 9H, 3x$CH_{3,}$ H-2$^{''''}$ , 3$^{''''}$ & 4$^{''''}$ ). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 152.76 (C, C-2), 119.59 (C, C-3), 152.76 (C, C-4), 104.49 (CH, C-5), 123.24 (C, C-6), 99.12 (CH, C-7), 156.34 (C, C-8), 114.01 (C, C-9), 123.77 (C, C-1$^{'}$), 133.62 (CH, C-2$^{'}$ & 6$^{'}$), 115.54 (CH, C-3' & 5$^{'}$), 163.53 (C, C-4$^{'}$), 56.24 ($CH_3$, C-7$^{'}$), 131.64 (C,C-1$^{''}$)'.105.14 (C, C-2$^{''}$& 6$^{''}$), 153.50 (C, C-3$^{''}$ &5$^{''}$), 139.22 (C, C-4$^{''}$), 56.24 ($OCH_3$, C-7'', 8$^{''}$, 9$^{''}$& 1$^{'''}$), 165.77 (CO, C-1$^{'''}$), 80.97 (C$_{,}$ C-1$^{''''}$), 29.65 ($CH_{3,}$ C-2$^{''''}$ , 3''''' & 4$^{''''}$). ESMS for $C_{29}H_{29}FO_7$ (MeOH): 509 $[M+H]^+$

***iso*-Pentanyl, 2-(4-fluorophenyl)-4-methoxy-3-(3,4,5-trimethoxyphenyl)benzofuran-6-carboxylate (9H):** Viscous compound. In $^1$H NMR (500MHz, $CDCl_3$): δ 7.14 (s, 1H, H-5), 7.70 (s, 1H, H-7), 7.52 (d, 2H, H-2$^{'}$ & 6$^{'}$ J= 8.5 Hz), 7.02 (d, 2H, H-3$^{'}$& 5$^{'}$ J= 9.0 Hz), 7.01 (s, 1H, H-2$^{''}$ ) 6.59 (s, 1H, H-6''), 3.95 (s, 9H, $OCH_3$, H-7'' , 9$^{''}$ & 1$^{'''}$), 4.00 (s, 3H , $OCH_{3,}$ H-8$^{''}$), 4.38 (t, 2H, $CH_2$, H-1$^{''''}$J= 6.0 Hz) 1.68 (m, 2H, $CH_2$, H-2$^{''''}$), 1.76 (m, 1H, CH, H-3$^{''''}$), 0.87 (s, 6H, 2X$CH_3$, H-4$^{''''}$ & H-5$^{''''}$). $^{13}$C NMR (125MHz, $CDCl_3$): ppm 143.17 (C, C-2), 115.36 (C, C-3), 160.78 (C, C-4), 104.40 (CH, C-5), 122.91 (C, C-6), 104.40 (CH, C-7), 160.78 (C, C-8), 115.18 (C, C-9), 122.09 (C, C-1$^{'}$), 130.93 (CH, C-2$^{'}$ & 6$^{'}$), 115.36 (CH, C-3' & 5$^{'}$), 166.11 (C, C-4$^{'}$), 130.93 (C,C-1$^{''}$)'.105.16 (C, C-2$^{''}$& 6$^{''}$), 160.78 (C, C-3$^{''}$ &5$^{''}$), 139.34 (C, C-4$^{''}$), 55.95 ($OCH_3$, C-7$^{''}$ , 8$^{''}$ , 9$^{''}$& 1$^{'''}$), 166.11 (CO, C-1$^{'''}$), 64.06 ($CH_{2,}$ C-1$^{''''}$) 37.08 ($CH_{2,}$ C-2$^{''''}$) 24.51 (CH$_{,}$ C-3$^{''''}$), 22.37 ($CH_{3,}$ C-4$^{''''}$ and 5$^{''''}$). ESMS for $C_{30}H_{31}FO_7$ (MeOH): 423 $[M+H]^+$

***sec*-Butyl-2-(4-fluorophenyl)-4-methoxy-3-(3,4,5-trimethoxyphenyl)benzofuran-6-carboxylate (9I):** Viscous compound. In $^1$H NMR (500MHz, $CDCl_3$): δ 7.19 (s, 1H, H-5$_{)}$, 7.54 (bs, 1H, H-7), 7.84 (d, 2H, H-2$^{'}$ & 6$^{'}$ J= 8.5 Hz), 7.00 (d, 2H, H-3$^{'}$& 5$^{'}$ J= 7.0 Hz), 6.93 (s, 1H, H-2$^{''}$ ) 6.94 (s, 1H, H-6''), 3.91 (s, 12H, $OCH_3$, H-7'', 8$^{''}$, 9$^{''}$ & 1'''), 1.43 (d, 3H, $CH_{3,}$ H-1$^{''''}$ J= 7.0 Hz), 4.24 (m, CH$_{,}$ 1H, H-2$^{''''}$ ), 1.92 (m, $CH_2$,H-3$^{''''}$ ), 0.82 (t, 3H, $CH_{3,}$ H-4$^{''''}$ J= 6.5 Hz ). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 153.09 (C, C-2), 119.48 (C, C-3), 153.09 (C, C-4), 106.03 (CH, C-5), 130.60 (C, C-6), 99.20 (CH, C-7), 156.34 (C, C-8), 113.62 (C, C-9), 123.54 (C, C-1$^{'}$), 131.10 (CH, C-2$^{'}$ & 6$^{'}$), 115.29 (CH, C-3' & 5$^{'}$), 163.57 (C, C-4$^{'}$), 133.55 (C, C-1$^{''}$)' 105.12 (C, C-2$^{''}$& 6$^{''}$), 153.09 (C, C-3$^{''}$ &5$^{''}$), 139.11 (C, C-4$^{''}$), 56.24 ($OCH_3$, C-7'', 9$^{''}$& 1$^{'''}$), 62.03 ($OCH_3$, C-8$^{''}$), 166.24 (CO, C-1$^{'''}$), 22.57 ($CH_{3,}$ C-1$^{''''}$), 65.32 (CH$_{,}$ C-2$^{''''}$), 29.58 ($CH_2$ , C-3'''''), 8.46 ($CH_3$, C-4$^{''''}$). ESMS for $C_{29}H_{29}FO_7$ (MeOH): 509 $[M+H]^+$

**Methyl-2-(4-methoxycarbonyl) phenyl)-4-methoxy-3-(3,4,5-trimethoxy phenyl) benzofuran-6-carboxylate (10A) :** Melting point: 150.4-160 $^0$C. In $^1$H NMR (500MHz, $CDCl_3$): δ 7.65 (s, 1H, H-5$_{)}$, 8.02 (s, 1H, H-7), ), 8.01 (d, 2H, H-2$^{'}$ & 6$^{'}$ J= 8.0 Hz), 7.64 (d, 2H, H-3$^{'}$& 5$^{'}$ J= 8.0 Hz), 7.24 (s, 1H, H-2$^{''}$ ) 6.25 (s, 1H, H-6''), 3.92 (s, 6H, $OCH_3$, H-7'' & 9$^{''}$), 3.94 (s, 3H , $OCH_{3,}$ H-8$^{''}$), 3.96 (s, 6H, $OCH_3$, H-1$^{''''}$ & 8$^{'}$). $^{13}$C NMR ($CDCl_3$, 125 MHz): ppm 142.85 (C, C-2), 115.90 (C, C-3), 151.89 (C, C-4), 108.93 (CH, C-5), 124.06 (C, C-6), 105.69 (CH, C-7), 161.17 (C, C-8), 123.51 (C, C-9), 124.06 (C, C-1$^{'}$), 129.42 (CH, C-2$^{'}$ & 6$^{'}$), 114.08 (CH, C-3' & 5$^{'}$), 131.36 (C, C-4$^{'}$), 52.25 ($CH_3$, C-7$^{''}$), 135.18 (C, C-1$^{''}$), 104.56 (C, C-2$^{''}$& 6$^{''}$), 151.89 (C, C-3$^{''}$ &5$^{''}$), 139.30 (C, C-4$^{''}$), 52.50 ($OCH_3$, C-7$^{''}$ , 8$^{'}$ & 9$^{''}$), 56.37 ($OCH_{3.}$ C-8$^{''}$), 52.25 ($OCH_3$, C-1$^{'''}$), 166.53 (CO, C-7$^{'}$), 166.67 (CO, C-1$^{'''}$), 52.34 ($OCH_{3,}$ C-1$^{''''}$). ESMS for $C_{28}H_{26}O_9$ (MeOH): 507 $[M+H]^+$