IN VITRO ANTIOXIDANT, ANTIMICROBIAL AND ADMET STUDY OF NOVEL FURAN/BENZOFURAN C-2 COUPLED QUINOLINE HYBRIDS

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INTRODUCTION

Free radicals are species capable of free existence that contains one or more unpaired electrons which react with another molecule by accepting or donating the electrons [1]. The harmful intervention of reactive oxygen species (ROS) in normal metabolic processes leads to pathologic changes which is a consequence of their interaction with biomolecules inside and outside the cells [2, 3]. ROS contain molecules like hydrogen peroxide (H2O2); hydroxyl radical (·OH) and superoxide (O2·−), by the generation of ROS, there is an alternative in the normal functioning of the cell and leads to pathophysiological changes. Free radicals are responsible for causing a wide number of health hazards such as cancer, ageing, heart diseases and gastric problems, etc. Oxygen free radicals disintegrate DNA and destroy cell membranes by enzymatic metabolic processes [4-6]. In nature’s collection of biologically active molecules, benzofuran derivatives constitute a major group [7-9]. Benzofuran ring systems bearing substitutions at the C-2 position are widely distributed in nature and have been proposed to have antioxidant, antiviral, antifungal activities [10, 11], antimicrobial [12, 13], anti-inflammatory [14], antipsychotic [15], analgesic [16], antilipidemic [17] and CNS stimulant activities [18]. Investigation of benzofuran derivatives in search of new drugs or to increase the efficacy of the present drugs has been most frequent approach [19]. Similarly, furan and its analogues were found to be biologically useful. 2-(furan-2-yl) quinoline-4-carboxylic acid and its analogues were reported to have the inhibiting property of C. albicans prohl-lRNA synthetase and showed potent in vitro antifungal activities against dermatophytes [20, 21]. 2-(furan-2-yl)-4-(phenoxyl) quinoline derivatives were screened for cytotoxicity and anti-inflammatory activities [22]. 4-[phenylamino]-furo[2, 3-b] quinoline and 2-[furan-2-yl]-4-(phenylamino) quinoline derivatives for cytotoxicity evaluation and 1-[4-(furo[2, 3-b] quinoline-4-yl) amino] phenyl ethanone exhibited potential and broad spectrum of cytotoxicity [23, 24]. The quinoline ring system is an essential structural fragment of a large number of natural and synthesized compounds displaying interesting biological activities such as antimalarial, antibacterial, anti-asthmatic, antihypertensive, and anti-inflammatory [25-27]. Quinolines and their derivatives have been found applications as pharmaceuticals and agrochemicals, as well as being general synthetic building blocks [28-31]. Molecular hybridization is a rational approach to design new prototypes after coupling different pharmacophoric subunits that can be recognized by two or more biologic receptors [32]. These strategies have been used in drug discovery to increase the efficacy.

In view of these observations and in continuation of our work on drug discovery through cinchophene and their derivatives [33-36], herein we report a facile, inexpensive procedure in the preparation of novel hybrid molecules [2-(1-benzofuran-2-yl) quinolin-4-yl] methanol and [2-(1-furan-2-yl) quinolin-4-yl] methanol derivatives using mild conditions, and explored for their in vitro antioxidant, antimicrobial and preliminary in silico ADMET properties.

MATERIALSANDMETHODS

Materials

Chemicals used in the synthesis of compounds were from Alfa Aesar, Pvt. Ltd. Bangalore, India and Spectrochem Pvt. Ltd. Bangalore, India. The solvents were of reagent grade and when necessary, they were purified by standard techniques.

Synthesis of novel 2-(benzofuran-2-yl) and 2-(furan-2-yl) quinoline-4-carboxylates and their [2-(1-benzofuran-2-yl) quinolin-4-yl] methanol, [2-(1-furan-2-yl) quinolin-4-yl] methanol and its derivatives for antioxidant, antimicrobial and in silico pharmacokinetic study.

Methods: Synthesis was carried with the conventional method and the structures were confirmed by IR, 1H NMR, 13C NMR and mass spectral analysis. The antioxidant activity was performed by DPPH and H2O2 radical scavenging method. The antimicrobial investigation was established by cup plate and food technique. The in silico absorption, distribution, metabolism, excretion and toxicity (ADMET) study of the drug was carried out in ACD/lab-2.

Results: The antioxidant activity results revealed that compounds 4b-c, 5a-b, 10c and 10f exhibited good DPPH radical and hydrogen peroxide scavenging activity. The antibacterial results revealed that compounds 4c, 5a-b, 10b, 10d and 10f exhibited good activity against Escherichia coli, Klebsiella pneumonia and Salmonella typhimurium. Further, the antifungal activity results showed that compounds 4c, 5c and 10c-e were showing good activity against Aspergillusflavus and Candida neoformans. The mean value of P<0.05 were considered to be statistically significant. The ADMET results revealed that compounds emerged as a potential candidate for antioxidant and antimicrobial agents.

Conclusion: The study reveals that compounds containing furan/benzofuran coupled heterocycles played the important role for activity as they possess potent antioxidant and antimicrobial agents. The in silico ADMET analysis also suggesting the compounds were in acceptable range to obey the pharmacokinetic parameters.

Keywords: DPPH, Lipinski, Toxicity, Escherichia coli and Aspergillusflavus
were purified and dried. Melting points of the synthesized compounds were determined with the help of Raga digital melting point apparatus and are uncorrected; Infrared data were recorded on a Bruker spectrophotometer using KBr pellets. 

**Methods**

**Synthesis of 1-(1-benzofuran-2-yl) ethanone (1)**

Synthesis of 1-(1-benzofuran-2-yl) ethanone was achieved by the addition of salicylaldehyde (5.8 g, 0.047 mol) chloroacetic acid (4.3 g, 0.047 mol) to an alcoholic KOH (33 %, 20 ml) solution and kept for about 2-3 h. The reaction mixture was cooled to room temperature and poured onto crushed ice. The separation was filtered and dried to get yellow amorphous powder, yield 80 %.

**General procedure for the synthesis of substituted 2-(1-benzofuran-2-yl) quinoline-4-carboxylate (4-a-c)**

A mixture of 1-(1-benzofuran-2-yl) ethanone (1.8 g, 0.0113 mol) and substituted 1H-indole-2, 3-dione (1.5 g, 0.0113 mol) in ethanol (10 ml) and an aqueous solution of KOH (33 %) 5 ml was added, the reaction mixture was stirred at 65-70 °C for about 12-14 h. The reaction mixture was poured onto crushed ice. The separated solid was filtered and recrystallized from petroleum ether (60-80). The yield was 85 %.

**General procedure for the synthesis of substituted methyl 2-(1-benzofuran-2-yl) quinoline-4-carboxylates (4-a-c)**

Analogs of 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acids were dissolved in sufficient quantity of methanol with a catalytic amount of Conc. H2SO4, and the mixture was refluxed for about 10-12 h. The reaction mixture was cooled to room temperature and poured onto the crushed ice, filtered, washed with water, dried and recrystallized from petroleum ether (60-80) and ethyl acetate (3:1:v).

**General procedure for the synthesis of substituted [2-(1-furan-2-yl) quinolin-4-yl] methanol (5a)**

To a 100 ml round bottom flask, dissolve methyl 2-(1-benzofuran-2-yl) quinoline-4-carboxylic acid in ethanol and maintain the reaction mixture below 5 °C, followed by the addition of Conc. H2SO4 and the resulting mass was filtered and dried to get yellow solid mass was filtered, washed with water, dried and recrystallized from petroleum ether (60-80). The yield was 79 %.

**General procedure for the synthesis of substituted 2-(1-furan-2-yl) quinoline-4-carboxylic acid (6)**

The compounds 8 (a-f) was synthesized by literature method [37] with slight modification. The reaction mixture was cooled to room temperature and poured onto crushed ice after neutralizing with 10 M HCl. The filtered residue was repeatedly washed with ethyl acetate (4-5 times).

**General procedure for the synthesis of substituted methyl2-(1-furan-2-yl) quinoline-4-carboxylates (9-a-c)**

Analogs of 2-(1-furan-2-yl) quinoline-4-carboxylic acids were dissolved in sufficient quantities of methanol with a catalytic amount of Conc. H2SO4 and was refluxed for about 12-14 h. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was cooled to room temperature and poured onto the crushed ice. The resulting solid mass was filtered, washed with water, dried and recrystallized from petroleum ether (60-80).
ADME-toxicity prediction were obtained by using ACD labs Chem sketch version 12.0. SMILES Chemical structures and SMILES notations of the title compounds were decided the safety and efficacy of active molecules take up the to predict the toxicity of lead molecules with intraperitoneal, oral, [39] and hepatotoxicity, Caco-2 cell permeability (QPPCaco) also help biological important endpoints. Aqueous solubility (PlogS), blood–brain barrier penetration (QkBB), intestinal absorption (logHIA) [39] and hepatotoxicity, Caco-2 cell permeability (QPPCaco) also help to predict the toxicity of lead molecules with intraperitoneal, oral, intravenous and subcutaneous toxic effects. The in silico study enables to decide the safety and efficacy of active molecules take up the molecule for in-depth studies.

Calculation of pharmacokinetic parameters and toxicity potential

Chemical structures and SMILES notations of the title compounds were obtained by using ACD labs Chem sketch version 1.20. SMILES notations of the derivatives were then fed in the online free version 2011.06 to calculate various molecular properties and to predict the bioactivity score for drug targets including enzymes and nuclear receptors, kinase inhibitors, GPCR ligands and ion channel modulators. Molecular properties such as partition coefficient (Log P), topological polar surface area (TPSA), hydrogen bond donors and acceptors, rotatable bonds, number of atoms, molecular weight, and violations of Lipinski’s rule of five were calculated to evaluate the drug-like-ness of the synthesized compounds [40]. The bioactivity score and drug-likeness properties of the title compounds were compared with the standard drugs streptomycin, ciprofloxacin and pyrazinamide respectively.

Biological evaluation

DPPH free radical scavenging assay

The assay was performed after modification of the method described by Blois [41]. 0.1 ml of different concentrations of amethanolic solution of standard and test compounds (0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml) was added to 2 ml of DPPH methanolic solution (60 mm). The mixture was shaken vigorously and allowed to react at room temperature and in darkness for 5 h. The absorbance of the resulting solution was measured at 517 nm using a UV/Vis spectrophotometer after 5 h incubation. Scavenging of DPPH free radicals was calculated as:

\[
\text{DPPH scavenging activity (\%)} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100
\]

Where Ac is the absorbance of the control tube [containing all reagents except the test compound], and At is the absorbance of the test tube. Ascorbic acid was used as the standard in the concentration range of 0.5-1.5 mg/ml.

Hydrogen peroxide scavenging assay

H₂O₂ scavenging power was determined according to the method of Ruch and co-workers [42]. The method is based on the ability of a compound to convert hydrogen peroxide to water. 40 nm solution of hydrogen peroxide was prepared in saline phosphate buffer (pH 7.4). 100 μl DMSO solutions of the test compounds or standards at the concentrations of 0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml were separately added to 2 ml of the prepared hydrogen peroxide solution and the absorbance was measured at 230 nm after 10 min against a blank solution. The hydrogen peroxide scavenging activity for compounds and standards was calculated using the following equation:

\[
\text{H}_2\text{O}_2\text{ scavenging activity (\%)} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100
\]

Where Ac is the absorbance of the control and At is the absorbance of the tested compounds or standards. Ascorbic acid at the concentration range of 0.5-1.5 mg/ml was used as the standard.

Antibacterial activity

Cup plate method

The antibacterial activity of synthesized molecules was studied systematically against three different strains of bacteria such as E. coli (ATCC No. 25922), K. pneumonia (ATCC No. 700603) and S. typhimurium (ATCC No. 14028) (gram-negative) by the agar diffusion method [43-45]. The organisms were subcultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37±1 °C for 24 h. They were stored in a refrigerator. The flasks with incubated bacterial inoculums were prepared by transferring a 10a-f) are predicted by pharmacokinetics parameters like absorption, distribution, metabolism, excretion and toxicity (ADMET). The ADMET/SAR [38] helps to evaluate biologically active molecules and eliminate a biologically poor molecule, an active lead molecule which contains undesirable functional groups based on Lipinski rule. The statistical calculation for lead molecules includes surface area, geometry and fingerprint properties which help to understand biological important endpoints. Aqueous solubility (PlogS), blood–brain barrier penetration (QkBB), intestinal absorption (logHIA) [39] and hepatotoxicity, Caco-2 cell permeability (QPPCaco) also help to predict the toxicity of lead molecules with intraperitoneal, oral, intravenous and subcutaneous toxic effects. The in silico study enables to decide the safety and efficacy of active molecules take up the molecule for in-depth studies.

Calculation of pharmacokinetic parameters and toxicity potential

Chemical structures and SMILES notations of the title compounds were obtained by using ACD labs Chem sketch version 1.20. SMILES pharmacokinetic parameter studies

ADME-toxicity prediction

The molecular descriptors of synthesized compounds [4a-c, 5a-c and 10a-f] are predicted by pharmacokinetics parameters like absorption, distribution, metabolism, excretion and toxicity (ADMET). The ADMET/SAR [38] helps to evaluate biologically active molecules and eliminate a biologically poor molecule, an active lead molecule which contains undesirable functional groups based on Lipinski rule. The statistical calculation for lead molecules includes surface area, geometry and fingerprint properties which help to understand biological important endpoints. Aqueous solubility (PlogS), blood–brain barrier penetration (QkBB), intestinal absorption (logHIA) [39] and hepatotoxicity, Caco-2 cell permeability (QPPCaco) also help to predict the toxicity of lead molecules with intraperitoneal, oral, intravenous and subcutaneous toxic effects. The in silico study enables to decide the safety and efficacy of active molecules take up the molecule for in-depth studies.

Calculation of pharmacokinetic parameters and toxicity potential

Chemical structures and SMILES notations of the title compounds were obtained by using ACD labs Chem sketch version 1.20. SMILES
Antifungal activity

Poisoned food technique was performed to investigate the antifungal effect of test compounds against Aspergillus flavus and Cryptococcus neoformans. Synthesized compounds were tested for their antifungal activity [46]. The fungi employed for screening were sub-cultured using potato dextrose agar medium. The potato-dextrose-agar medium was sterilized by autoclave at 121 °C (15 lb/sq. inch), for 15 min. The petri-plates, tubes and flasks plugged into the nutrient agar medium. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1 ml of dimethyl sulphoxide to observe the solvent effects. After incubation of the plates at 37±1 °C for 24 h, the diameter of the zone of inhibition was read with the help of antibiotic zone scale.

Antifungal activity

The experiments were performed in triplicate in order to minimize the error. The inhibition percentage of the A. flavus and C. neoformans mycelial growth was calculated using the following formula.

\[ IP = 100 \times \frac{CT}{C} \]

Where C is the average of 3 replicates of mycelial growth (cm) of control petri dishes and T is the average of 3 replicates of mycelial growth (cm) of treated petri dishes. Fluconazole was used as the positive control and DMSO was thus used as the negative control.

**RESULTS AND DISCUSSION**

**Chemistry**

The synthesis of key intermediate 1-(1-benzofuran-2-yl)ethanone (1) and its utilization in the construction of 2-(1-benzofuran-2-yl)quinoline-4-carboxylic acid 3(a-c) and 8(a-f) were shown in Scheme-I, II and Table 1. The intermediate (1) was synthesized by stirring a mixture of 2-hydroxybenzaldehyde with chloroacetonitrile in basic medium using methanol as solvent. Subsequently, 2-(1-benzofuran-2-yl)quinoline-4-carboxylic acids 3(a-c) were synthesized by the reaction of a compound (1) with substituted isatins 2(a-c) in basic medium to obtain the products 3(a-c). The ester, methyl 2-(1-benzofuran-2-yl)quinoline-4-carboxylates 4(a-c) were synthesized by reacting 3(a-c) with methyl alcohol in the presence of catalytic amount of Conc. H\textsubscript{2}SO\textsubscript{4} to obtain the product in good yield. The alcohol 5(a-c) was synthesized by the reaction of compound 4(a-c) with reducing agent sodium borotetrahydride, under stirring condition.

**Table 1: Particulars of the derivatives of [2-(1-benzofuran-2-yl)quinolin-4-yl] methanol and [2-(1-furan-2-yl)quinolin-4-yl] methanol**

| S. No. | Samples code | R  | Molecular formula | Molecular weight | (%)/yield | Melting point (°C) |
|--------|--------------|----|-------------------|------------------|-----------|-------------------|
| 1      | 4a           | -H-| C\textsubscript{11}H\textsubscript{14}N\textsubscript{3}O\textsubscript{3} | 303.31           | 83        | 115-118           |
| 2      | 4b           | -Cl-| C\textsubscript{11}H\textsubscript{14}N\textsubscript{3}O\textsubscript{3} | 337.75           | 85        | 125-128           |
| 3      | 4c           | -F-  | C\textsubscript{11}H\textsubscript{14}F\textsubscript{3}N\textsubscript{3}O\textsubscript{3} | 301.30           | 80        | 121-124           |
| 4      | 5a           | -H-  | C\textsubscript{11}H\textsubscript{14}F\textsubscript{3}N\textsubscript{3}O\textsubscript{3} | 275.30           | 78        | 154-156           |
| 5      | 5b           | -Cl- | C\textsubscript{11}H\textsubscript{14}F\textsubscript{3}N\textsubscript{3}O\textsubscript{3} | 309.00           | 81        | 206-208           |
| 6      | 5c           | -F-  | C\textsubscript{11}H\textsubscript{14}F\textsubscript{3}N\textsubscript{3}O\textsubscript{3} | 293.00           | 79        | 158-160           |
| 7      | 10a          | -H-  | C\textsubscript{15}H\textsubscript{15}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{2} | 259.68           | 88        | 148-150           |
| 8      | 10b          | -H-  | C\textsubscript{15}H\textsubscript{15}Br\textsubscript{2}N\textsubscript{2}O\textsubscript{2} | 304.13           | 83        | 151-153           |
| 9      | 10c          | -F-  | C\textsubscript{15}H\textsubscript{15}F\textsubscript{2}N\textsubscript{2}O\textsubscript{2} | 243.23           | 82        | 85-87             |
| 10     | 10d          | -CH\textsubscript{3} | C\textsubscript{15}H\textsubscript{15}Cl\textsubscript{2}N\textsubscript{2}O\textsubscript{2} | 273.71           | 77        | 136-138           |
| 11     | 10e          | -CH\textsubscript{3} | C\textsubscript{15}H\textsubscript{15}Br\textsubscript{2}N\textsubscript{2}O\textsubscript{2} | 318.16           | 81        | 104-106           |
| 12     | 10f          | -CH\textsubscript{3} | C\textsubscript{15}H\textsubscript{15}F\textsubscript{2}N\textsubscript{2}O\textsubscript{2} | 257.25           | 78        | 118-120           |

**IR spectra of the compounds showed an absorption band in the range of 3114 cm\(^{-1}\) due to the characteristic-OH group stretching. The \(^1\)H NMR spectra of 5a showed a singlet at δ 5.25 ppm corresponding to methyl proton of \[\text{quinolin-4-yl}\] methanol, a singlet at δ 1.72 ppm corresponding to alcohol proton of methyl alcohol and the remaining peaks which appeared at δ 8.20 to 7.26 ppm corresponding to aromatic protons of the quinoline and benzofuran rings. The \(^1\)C-NMR spectra of 5a showed a characteristic peak at δ 67.0 ppm corresponding to the methyl carbon of \[\text{quinolin-4-yl}\] methanol, and the peaks between δ 159.1 to 1085 ppm corresponding to aromatic carbons. MS analysis of 5(a-c) displayed their corresponding molecular ion peak confirming their molecular weight.**

**Scheme I: Synthesis of substituted [2-(1-benzofuran-2-yl)quinolin-4-yl] methanol 5(a-c)**
The peaks between δ 8.08 to 6.59 ppm corresponding to benzofuran and quinoline ring protons. The 13C-NMR spectra of 10a showed a peak at δ 59.7 ppm corresponding to the methyl carbon of [quinolin-4-yl] methanol, and the peaks between δ 152.9 to 110.7 ppm corresponding to aromatic carbons. MS analysis of 10a displayed the molecular ion peak confirming their molecular weight. The 1H NMR spectra of 10d showed a singlet at δ 5.14 ppm corresponding to methyl protons of [quinolin-4-yl] methanol, it showed singlet at δ 2.46 ppm corresponding to furan substituted methyl protons and appeared a singlet at δ 1.81 ppm corresponding to the alcohol proton of [quinolin-4-yl] methanol and the aromatic peaks which appeared between δ 8.05 to 6.19 ppm. The 13C-NMR spectra of 10d showed a peak at δ 59.7 ppm corresponding to the methyl carbon of [quinolin-4-yl] methanol, peak at δ 13.6 ppm corresponding to the proton of [quinolin-4-yl] methanol and the aromatic peaks which appeared a singlet at δ 1.81 ppm corresponding to the alcohol proton of [quinolin-4-yl] methanol, and the peaks between δ 152.9 to 110.7 ppm corresponding to aromatic carbons.

**In silico** ADMET (Absorption, distribution, metabolism, excretion and toxicity) profile

The compounds with poor bioavailability show less effectiveness against disease. To overcome this problem, predicting bioavailability properties will be of great advantage in drug development. Hence, using computer-based methods like ADMET and SAR tools the molecular descriptors and drug likeness properties was studied. The pharmacokinetic properties are represented in table 2 and 3. The in silico data of all the molecules displayed are within acceptable range. The interpretation of test compounds with reference standards (streptomycin, fluconazole and ascorbic acid) show that the compounds 4a-c, 5a-c and 10a-f were in good, acceptable range and hence, further used to make an oral formulation for absorption and to transport proteins and metabolizing enzymes to maintain homeostatic condition. The intestinal absorption (logD50) and Caco-2 cell permeability (PCaco-2) within the range of 2 poor absorption and+2 more absorption reveal that the compounds are more permeable in the intestine and helps for good transport of the drug or its metabolites.

The reference range of 5 (poor) to 1 (good) and substrate inhibitor from 0 to 1 in which the reference and test compounds 4a-c, 5a-c and 10a-f shows significant activity with human intestinal absorption and metabolism. The aqueous solubility of compounds lies with a range of 0 (poor) to 2 (good) showed that all the molecules have good solubility. While the reference compound, as well as test compounds, came within the acceptable range (table 3).

The toxicity of the substituted [2-(1-benzofuran-2-yl) quinolin-4-yl] methanol and [2-(1-furan-2-yl) quinolin-4-yl] methanol was predicted. The probability of health effects was predicted using ACD/I-Lab 2.0 (guest). The toxicity of selected compounds was listed in table 2. The LD50 of potential compounds detects the cumulative potential of acute toxicity that is administered through oral, subcutaneous, intra peritoneal, intravenous and subcutaneous on mouse models. The comparative analysis of reference compounds with test compounds on subcutaneous, intra-peritoneal, oral and intravenous is low when compared to the reference molecule. The toxicity results suggest that the compounds 4a-c, 5a-c and 10a-f have less toxic effect to tissue and with no side effect (table 2). Hence, can be considered for further development.

| Ligands | Intraperitoneal+ | Oral+ | Intravenous+ | Subcutaneous+ |
|---------|-----------------|-------|--------------|---------------|
| 4a      | 490(0.45)       | 540(0.15) | 55(0.49)      | 490(0.28)     |
| 4b      | 500(0.4)        | 450(0.18) | 56(0.48)      | 550(0.33)     |
| 4c      | 640(0.46)       | 460(0.15) | 50(0.47)      | 940(0.34)     |
| 5a      | 420(0.34)       | 840(0.32) | 46(0.47)      | 620(0.25)     |
| 5b      | 400(0.34)       | 810(0.28) | 42(0.47)      | 520(0.28)     |
| 5c      | 620(0.44)       | 690(0.32) | 47(0.47)      | 570(0.29)     |
| 5d      | 260(0.43)       | 800(0.25) | 65(0.4)       | 470(0.38)     |
| 5e      | 280(0.36)       | 870(0.34) | 67(0.35)      | 770(0.33)     |
| 10a     | 380(0.28)       | 550(0.29) | 78(0.38)      | 760(0.33)     |
| 10b     | 260(0.44)       | 780(0.23) | 62(0.39)      | 500(0.39)     |
| 10c     | 250(0.37)       | 840(0.34) | 59(0.35)      | 720(0.34)     |
| 10d     | 350(0.29)       | 580(0.34) | 67(0.38)      | 680(0.32)     |
| Streptomycin | 310(0.76) | 880(0.53) | 110(0.67)     | 400(0.52) |
| Fluconazole | 1200(0.73) | 1000(0.51) | 58(0.47)  | 2700(0.23)  |
| Ascorbic acid | 1100(0.7) | 4500(0.61) | 820(0.58) | 2700(0.5) |

Estimated LD50-mouse value in mg/kg after Intraperitoneal+, Oral+, Intravenous+ and Subcutaneous+ administration. The drugs with amoderate effect on reliability index (>0.5) The drugs with borderline effect on reliability index (>0.3,<0.5).
Antioxidant studies

The antioxidant potential of synthesized compounds (4a-c, 5a-c and 10a-f) was determined as an index of pharmacological usefulness. Two in vitro models were used to evaluate antioxidant properties; radical scavenging and hydrogen peroxide scavenging activity. The antioxidant properties were expressed in terms of percentage inhibition and 50 percent inhibitory concentration (IC50) values (table Sand6).

DPPH radical scavenging assay

The antioxidant potential of compounds (4a-c, 5a-c and 10a-f) was determined by in vitro model systems(Blois 1958) and were used for the evaluation of antioxidant properties, namely, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and expressed in terms of 50% inhibitory concentration (IC50) values (table 6).
The DPPH radical scavenging assay is one of the best free radicals scavenging mechanism (Scheme-III) by which antioxidant inhibits the oxidation and offer a rapid technique for screening the radical scavenging activity of specific compounds. All tested molecules exhibited a certain degree of radical scavenging activity. The compounds 4c, 5b, 10c and 10f exhibited good radical scavenging potential (table 5) compared with standard, ascorbic acid.

**Hydrogen peroxide (H$_2$O$_2$) scavenging capacity assay**

The hydrogen peroxide scavenging ability of synthesized derivatives was determined according to the method of Ruch and co-workers 1989 [42].

A solution of H$_2$O$_2$ (40 mm) was prepared in phosphate buffer (pH 7.4).

### Table 5: Antioxidant activity of substituted [2-(1-benzofuran-2-yl) quinolin-4-yl] methanol and [2-(1-furan-2-yl) quinolin-4-yl] methanol derivatives (4a-c, 5a-c and 10a-f)

| Compounds code | DPPH scavenging activity % inhibition | H$_2$O$_2$ scavenging activity % inhibition |
|----------------|--------------------------------------|------------------------------------------|
| 4a             | 68±0.57                              | 64±1.0                                  |
| 4b             | 71±1.0                               | 70±0.57                                 |
| 4c             | 82±1.52                              | 68±0.57                                 |
| 5a             | 65±1.52                              | 69±1.0                                  |
| 5b             | 74±1.0                               | 70±2.5                                  |
| 5c             | 63±1.72                              | 58±1.0                                  |
| 10a            | 56±1.52                              | 52±3.02                                 |
| 10b            | 46±1.0                               | 45±1.52                                 |
| 10c            | 85±1.52                              | 72±1.72                                 |
| 10d            | 57±1.0                               | 52±0.57                                 |
| 10e            | 61±1.15                              | 64±1.52                                 |
| 10f            | 79±1.52                              | 69±2.64                                 |
| Ascorbic acid  |                                      | 96±1.15                                 |

Values are expressed as mean±SD (n=3). Values are significant with each other at P<0.05 (Duncan’s multiple range test).

### Table 6: IC$_{50}$ (µg/ml) values of DPPH and H$_2$O$_2$ scavenging activity of substituted [2-(1-benzofuran-2-yl) quinolin-4-yl] methanol and [2-(1-furan-2-yl) quinolin-4-yl] methanol analogues (4a-c, 5a-c and 10a-f)

| Compounds code | DPPH scavenging activity IC$_{50}$ (µg/ml) | H$_2$O$_2$ scavenging activity IC$_{50}$(µg/ml) |
|----------------|------------------------------------------|---------------------------------------------|
| 4a             | 16.8±0.57                                | 16±1.52                                    |
| 4b             | 17.4±0.25                                | 15.5±0.20                                  |
| 4c             | 20±1.0                                   | 16±1.15                                    |
| 5a             | 16.5±0.2                                 | 15±1.0                                     |
| 5b             | 18±1.15                                  | 14.5±0.23                                  |
| 5c             | 16.5±0.2                                 | 9.8±0.11                                   |
| 10a            | 11.5±0.23                                | 11±0.20                                    |
| 10b            | 9.5±0.20                                 | 8.2±1.15                                   |
| 10c            | 24±0.57                                  | 18±1.0                                     |
| 10d            | 12±1.52                                  | 11.5±0.20                                  |
| 10e            | 16±1.15                                  | 15±1.52                                    |
| 10f            | 13.8±0.15                                | 13.2±0.30                                  |
| Ascorbic acid  | 6±1.52                                   | 7.5±0.20                                   |

Values are expressed as mean±SD (n=3). Values are significant with each other at P<0.05 (Duncan’s multiple range test).
The solutions of different molecules (10-50 μg/ml) were prepared in phosphate buffer and were added to H₂O₂ solution (0.6 ml, 40 mm). The absorbance value of the reaction mixture was recorded at 230 nm. The blank solution contains phosphate buffer without H₂O₂. The percentage of an H₂O₂ scavenging of the entailed molecule and the standard compound was calculated as H₂O₂ radical scavenging activity.

\[ \text{scavenging} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]

Where

- \( A_0 \) is the absorbance of H₂O₂ solution
- \( A_1 \) is the absorbance of H₂O₂ solution in the presence of benzofuran/furan quinolone methanols.

Whereas the absorbance of H₂O₂ solution was calculated as H₂O₂, the absorbance of the blank solution contains phosphate buffer without H₂O₂, while the compounds 4b, 5a, 10c-d and 10f showed moderate activity. The compounds 4a-b, 5a-b, 10a-b and 10e-f had showed significant activity when compared with standard drug fluconazole. The results indicated that among the tested compounds, concerning antibacterial activities, compounds 4b, 5a-c and 10a-c and 10c-d were found to be potent against E. coli, while the compounds 4a-b, 5c, 10a, 10c and 10f showed significant activity. The compounds 4a, 5a and 10c-d found to be potent against K. pneumonia, while the compounds 4b-c, 5b-c, 10a-b and 10f showed moderate activity. The compounds 4c, 5a and 10c-d found to be possessed good inhibition against S. typhirium, while the compounds 4a-b, 5b-c, 10a-b and 10f have shown significant activity as compared with standard drug streptomycin. From the results, it could be accomplished that, the chloro and fluoro functioned derivatives showed better activity than unsubstituted derivatives against all bacterial strains.

### Table 7: Antimicrobial activities of substituted [2-(1-benzofuran-2-yl) quinolin-4-yl] methanol and [2-(1-furan-2-yl) quinolin-4-yl] methanol analogues (4a-c, 5a-c and 10a-f)

| Samples code | E. coli (%) | K. pneumonia (%) | S. typhirium (%) |
|--------------|-------------|------------------|------------------|
| 4a           | 3.1±0.25    | 3.0±0.12         | 3.1±0.12         |
| 4b           | 3.0±0.18    | 3.0±0.12         | 3.0±0.12         |
| 4c           | 3.1±0.12    | 3.0±0.12         | 3.1±0.12         |
| 5a           | 3.4±0.07    | 3.3±0.07         | 3.4±0.07         |
| 5b           | 3.3±0.17    | 3.2±0.12         | 3.3±0.17         |
| 5c           | 3.4±0.07    | 3.3±0.07         | 3.4±0.07         |
| 10a          | 3.5±0.12    | 3.4±0.12         | 3.5±0.12         |
| 10b          | 3.4±0.17    | 3.3±0.12         | 3.4±0.17         |
| 10c          | 3.4±0.17    | 3.3±0.12         | 3.4±0.17         |
| 10d          | 3.2±0.25    | 3.1±0.12         | 3.2±0.25         |
| 10e          | 3.2±0.25    | 3.1±0.12         | 3.2±0.25         |
| 10f          | 3.2±0.25    | 3.1±0.12         | 3.2±0.25         |
| Streptomycin  | 3.2±0.12    | 3.1±0.12         | 3.2±0.12         |

Values are expressed as mean±SD (n=3). Values are significant with each other at P<0.05 (Duncan’s multiple range test).

### Table 8: Antifungal activities of substituted [2-(1-benzofuran-2-yl) quinolin-4-yl] methanol and [2-(1-furan-2-yl) quinolin-4-yl] methanol analogues (4a-c, 5a-c and 10a-f)

| Samples code | A. flavus (% inhibition) | 50 µg/ml | 100 µg/ml | C. neoformans (% inhibition) | 50 µg/ml | 100 µg/ml |
|--------------|--------------------------|----------|-----------|-------------------------------|----------|-----------|
| 4a           | 3±0.12                   | 60±1.0   | 54±1.52   | 86±2.08                       | 80±0.57  | 80±0.57   |
| 4b           | 45±1.52                  | 68±1.0   | 56±3.5    | 80±0.57                       | 80±0.57  | 80±0.57   |
| 4c           | 56±1.0                   | 79±0.57  | 36±1.15   | 85±1.0                        | 85±1.0   | 85±1.0   |
| 5a           | 42±1.52                  | 70±2.08  | 48±1.52   | 76±1.0                        | 87±1.15  | 87±1.15   |
| 5b           | 47±1.0                   | 78±2.0   | 54±1.15   | 84±1.52                       | 84±1.52  | 84±1.52   |
| 5c           | 56±1.52                  | 68±0.57  | 41±1.0    | 84±1.52                       | 84±1.52  | 84±1.52   |
| 10a          | 34±1.0                   | 65±1.52  | 54±2.0    | 90±1.52                       | 86±3.0   | 86±3.0   |
| 10b          | 41±2.0                   | 70±1.15  | 50±1.52   | 86±3.0                        | 86±3.0   | 86±3.0   |
| 10c          | 51±3.5                   | 68±2.0   | 35±1.15   | 71±2.08                       | 71±2.08  | 71±2.08   |
| 10d          | 53±1.52                  | 74±2.0   | 54±3.5    | 86±3.0                        | 86±3.0   | 86±3.0   |
| 10e          | 57±1.0                   | 78±1.52  | 66±0.57   | 80±2.0                        | 80±2.0   | 80±2.0   |
| 10f          | 46±1.15                  | 82±2.0   | 44±3.5    | 74±1.73                       | 74±1.73  | 74±1.73   |
| Fluconazole  | 47±0.57                  | 64±2.3   | 42±0.57   | 75±0.57                       | 75±0.57  | 75±0.57   |

Values are expressed as mean±SD (n=3). Values are significant with each other at P<0.05 (Duncan’s multiple range test).

The compounds 4a-b, 5a-b, 10a-b and 10d showed good activity against C. reinformans, while the compounds 4c, 5c, 10c and 10f have showed significant activity when compared with standard drug fluconazole. The results exhibited that, the halogen-substituted derivatives showed better activity compared to other analogues.

### Antifungal activity

Further, the synthesized compounds were screened for their in vitro antifungal activity against A. flavus and C. reinformans because of their transmittable nature. The compounds were dissolved in DMSO and antifungal activity was determined by the poisoned food technique at concentrations of 50 and 100 μg/ml.

The antifungal result data (table 8) indicated that the synthesized compounds showed a variable degree of inhibition against fungal species, the compounds 4c, 5c and 10c-e showed potent activity against A. flavus, while the compounds 4a-b, 5a-b, 10a-b and 10f have shown moderate activity.

### Statistical analysis

The measurements were expressed as mean±SD for standard drugs. The data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT) by using statistical package of social science (SPSS) version 10.0 for
The present work reports the synthesis, spectral characterization and biological activities including antioxidant and antimicrobial activities of synthesized series of benzofuran/furan quinoline methanol analogues (table 1). Molecules were made to predict ADMET/SAR in silico, the synthesized molecules are in an acceptable range were further screened for antioxidant analysis with DPPH and H₂O₂ scavenger methods and antimicrobial activity. The work indicates that the entitled compounds were found to possess good antioxidant and potent antimicrobial activity. The potent molecules (4b-c, 5a-c, and 10b-f) will be further taken up for in detail study, by varying the functionalities on the aromatic rings with change in carbon chain length, the electron donor and withdrawing groups. The furan quinoline methanols can be considered as lead moieties for drug finding. The results provide useful information for operating as a positive reinforcement of the tendency to use antioxidant and antimicrobial properties as a guideline of the rational design of this class of compounds. It needs further detailed investigations such as in vivo pharmacokinetic profile, toxicity, the mechanism(s), are needed to evaluate their potential of developing into the therapeutic agents.

AUTHORS CONTRIBUTION

Dr. N. D. Satyanarayan is the mentor involved in designing and is the supervisor of the overall work. Mr. Anantacharya R is mainly involved in synthesis/characterization of entitled molecules and carried out in silico pharmacokinetic studies and their interpretation. Dr. K.M. Mahadevan involved in the idea generation of in the generation of new coupled heterocyclic analogues; Mr. Sameer P. was instrumental in carrying out in vitro antimicrobial and antioxidant results. Mr. Adarsha is helped in generating the spectral data.

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CONFLICT OF INTERESTS

There is no any conflict of interest

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