Chapter 2

Synthesis and biodynamic properties of benzofuran scaffolds
Chapter 2  Ben:ofuran Scaffold: Synthesis and Biodynamic properties

2.1 Introduction

Medicine and natural products have been closely linked through the use of traditional medicines and natural poisons for thousands of years. Clinical, pharmacological, and chemical studies of these traditional medicines derived predominantly from plants, were the basis of most early medicines. The plants belonging to genus *Tephrosia* Pers. and *Pongamia* Vent, are traditional Indian medicinal plants native to Western Ghats and chiefly found in tidal forests of India. The different parts of the plant have been used in the traditional system of the medicine for bronchitis, whooping cough, rheumatic joints and to quench dipsia in diabetes. Phytochemical examination of these plants indicated the presence of various furanoflavones, furanochalones, furanoflavonols, chromeneflavanones and furanodiketones, which possess diverse biological activities.

\[
\text{Lanceolatin B: } R_1 = R_2 = R_3 = H \\
\text{Isopongaglabol methyl ether: } R_1 = \text{OMe}, R_2 = R_3 = H \\
\text{Pongaglabrone: } R_1 R_2 = \text{OCH}_2 \text{O}, R_3 = H \\
\text{Pongol: } R_1 = R_3 = H, R_2 = \text{OH} \\
\text{Karanjonol: } R_1 = R_3 = H, R_2 = \text{OH} \\
\text{Karanjin: } R_1 = R_2 = H, R_3 = \text{OMe} \\
\text{Pongapin: } R_1 R_2 = \text{OCH}_2 \text{O}, R_3 = \text{OMe} \\
\text{Glabone: } R_1 = \text{OMe}, R_2 = R_3 = H \\
\text{Pongone: } R_1 = R_3 = H, R_2 = \text{OMe} \\
\text{Ponganone XI: } R_1 = R_2 = H, R_3 = \text{OMe} \\
\text{Pongamol: } R_1 = R_2 = H, R_3 = \text{OH} \\
\text{Ovalitenone: } R_1 R_2 = \text{OCH}_2 \text{O}, R_3 = \text{OH} \\
\text{Ovalitenin A: } R_1 = R_2 = R_3 = H \\
\text{Ovalitenin C: } R_1 R_2 = \text{OCH}_2 \text{O}, R_3 = \text{H} \\
\text{Purpuritenin: } R_1 = \text{CH}_3, R_2 = R_3 = \text{H}
\]

Figure 1. Natural products isolated from *Tephrosia* Pers. and *Pongamia* Vent

Chalcones and flavonoids are group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. The average human diet contains about 1g of flavonoids per day, assimilated through fruits, vegetables, red wine, tea etc. Most of the chalcones and flavonoids have been isolated from plants in order to test their biological properties; others were synthetically produced in search of more potent drug candidates. Over the last few decades extensive work has been done on the isolation and characterization of natural products derived from various species to uncover the chemical and biological properties. Several angular and linear furanoflavones and furanochalones have been
isolated from these plants (Figure 1) and displayed diverse biological activities such as anticancer, antiulcer, antiallergic, insecticidal, pesticidal, and antihelminthic.

Although these compounds possess immense biological potential but isolation of these compounds in small quantities limits the scope for further exploration. Therefore highly efficient, concise synthetic approach that could offer flexibility of substituents variations is highly desirable. Diverse pharmacological activities associated with these natural products and limitation of availability of these scaffolds from natural sources prompted us to develop a novel approach to the synthesis of naturally mimicking benzofuran methyl ketones (I) and their dimers (VII), naturally occurring various hydroxy furanoflavones and chalcones/furanochalcones (IV-VI) and to evaluate their biodynamic properties these compounds were screened for PTP-1B inhibitory activity for the treatment of diabetes, antileishmanial activity, antifungal activity and anti-tubercular activity.

2.2 Synthesis of 1-(5-alkoxy-2-hydroxyphenyl)-ethanone and their hydroxy chalcones (4a-f)

Several natural and synthetic compounds with acetophenones scaffold are biologically relevant. Umezawa et al. discovered a naturally occurring PTPase inhibitor, dephostatin isolated from the culture filtrate of Streptomyces sp. MJ742-NF5. Later on, they prepared several stable alkyl 3,4-dephostatin (VIII) as stable, selective inhibitor of PTP-1B (Figure 2). Recently, Pei et al. have reported several α-haloacetophenone (IX) derivatives as potent neutral protein tyrosine phosphatase inhibitors, which covalently alkylate the conserved catalytic cysteine residue in the PTP active site. Several formyl chromone (X) derivatives
were reported as human PTP-1B inhibitory activity in micromolar range.\textsuperscript{8} Recently Hu \textit{et al.}\textsuperscript{9} have shown that the ethanolic extract of the roots of \textit{Broussonetia papyrifera} (L.) Vent, which comprised of several flavonoids including a flavonol \textit{XI} showed potent inhibitory activity against PTP-1B enzyme.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structures}
\caption{Structures of PTP-1B inhibitors with acetophenones scaffold}
\end{figure}

Based on the reported PTP-1B inhibitors, we have synthesized various small molecule acetophenones functionalized with alkoxy and aroyloxy moieties as potential PTP-1B inhibitors. The precursor used for the synthesis of various hydroxy chalcones and functionalized acetophenones was 2,4-dihydroxyacetophenone 1, which on base catalyzed
alkylation afforded 2 in 60-70% yield as depicted in Scheme 1. The aroylation of 2 with aryl chloride in pyridine gave product 3 in good yields. The benzoylated acetophenone 3 on stirring with t-BuOK in pyridine (Baker-Venkataraman rearrangement) yielded hydroxychalcones 4 in 70-85% yield. All the synthesized compounds were characterized by spectroscopic analysis and evaluated for their PTP-1B inhibitory activity, antifungal activity and also for anti tubercular activity.

2.3 Synthesis of naturally occurring furanochalcones, angular and linear furanoflavones. (7a-e, 8a-e, 9a-d & 10a,b)

A key intermediate required for the synthesis of various naturally occurring furanochalcones and angular furanoflavones (Figure 1) is 5-acetyl-4-hydroxycoumarone (5) while 5-acetyl-6-hydroxycoumarone (6) is a precursor for linear furanoflavones. Although these coumarones are structurally very simple, they have been prepared in a minimum four steps in moderate yields (Figure 3). The literature procedures for the synthesis of 5-acetyl-4-hydroxycoumarone (5) include fission of natural products (pongamol and lanceolatin B); cyclization of 5-benzyloxy-2-formylphenoxyacetic acid ester followed by decarboxylation, catalytic reduction, acetylation and dehydrogenation; and allylation of resacetophenone followed by Claisen migration, and oxidative cyclization to yield the desired compounds in moderate yields. These procedures either require long isolation processing of natural products or multiple synthetic stages with recoveries of intermediates, harsh reaction conditions or undesired side products.

Recently, Lee et al. have developed a new method for the synthesis of 5-acetyl-4-hydroxycoumarone (5), which includes the reaction of diazocyclohexane-1,3-dione with vinyl acetates in presence of rhodium acetate followed by dehydration, acetylation and aromatization to yield 5-acetyl-4-hydroxycoumarone (5) in moderate yield. The desired starting materials can also be prepared through a well-established Dötz benzannulation approach, which includes heteroannulation using α,β-unsaturated chromium-carbene complexes and suitably functionalised alkynes. But, these procedures suffer with the
limited use of expensive metal complexes, tedious work-up for intermediates and low yield of the desired compound. These difficulties in obtaining a desired starting material limit the scope of derivatization of several natural or synthetic furanochalcones and furanoflavones.

The angular furanoflavone, lanceolatin B has been prepared by demethylation of naturally derived pongamol followed by acid-catalyzed intramolecular cyclization. Recently, the total synthesis of few angular furanoflavones have been achieved by preparing highly activated benzylidene-β-ketosulfoxide followed by conjugate addition of hydroxybenzaldehyde and intra-molecular cyclization in six steps in approximately 35% overall yield.

Other methods for preparing furanoflavones such as pongaglabrone were achieved by Seshadri et al. and Hossain starting from resacetophenone in a minimum of five steps. We developed a four-step process for the total synthesis of naturally occurring two angular furanoflavones: lanceolatin B, isopongaglabol methyl ether; two linear furanoflavones: glabone, pongone, and two furanochalcones: pongamol and ovalitenone. Other furanoflavones and furanochalcones can be prepared similarly using these coumarones.

Our approach to synthesize furanoflavones and furanochalcones is very simple and straightforward (Scheme 2). A reaction of resacetophenone (1) and bromoacetaldehyde diethyl acetal in the presence of anhydrous potassium carbonate in dry DMF delivered 1-[4-(2,2-diethoxy-ethoxy)-2-hydroxyphenyl]-ethanone (2f) in 90% yield.

Although many examples of cyclization of phenoxyacetals to coumarones are reported in the literature using various Lewis acids (SnCl4, AlCl3, BF3, ZnCl2) and organic acids (H2SO4, TFA, H3PO4, PPA, HCOOH, p-toluene sulfonic acid), but majority of them leads to extensive decomposition of the starting material leading to polymerized resinous material containing mixture of compounds. As the cyclization of phenoxyacetals is extremely dependent on the presence of substituents on the phenyl ring and the conditions employed. We attempted the cyclization of 4-(2,2-diethoxy-ethoxy)-2-hydroxyacetophenone (2f) to corresponding coumarones using BF3, ZnCl2, TFA, H3PO4, PPA, HCOOH. To the best of our efforts, only PPA mediated reaction conditions in aprotic solvents gave moderate yield of desired coumarones. Rest of the reaction conditions ended with only polymeric products. Then, we thought that an acidic resin A-15 (a macro reticular sulfonic acid based polystyrene cation exchange resin), which has been used to hydrolyze the acetals to their corresponding carbonyl compounds, might give the desired coumarone in sufficient yield.
This possibility was explored by carrying out the cyclization reaction of 2f in reflux toluene with A-15. The reaction was monitored by TLC, which took place in 8 hrs. The mixture was cooled to room temperature and filtered to remove the resin. The resulting mixture was concentrated under reduced pressure. The crude material thus, obtained was purified by silica gel column chromatography. Two products, 5-acetyl-4-hydroxycoumarone (5) and 5-acetyl-6-hydroxycoumarone (6) were isolated as minor products together with resinous substance as a major constituent, similar to the polymeric compound obtained by various acid catalyzed cyclizations.

The $^1$H NMR of the 5-acetyl-4-hydroxycoumarone (5) showed two singlets at $\delta$ 2.66 and 13.28 for acetyl and hydroxyl protons and four doublets at $\delta$ 7.00, 7.04, 7.57, and 7.66 for H-3, H-7, H-2 and H-6 protons respectively. This is in confirmation with the proposed angular structure of 5-acetyl-4-hydroxycoumarone (5).
The $^1$H NMR of the 5-acetyl-6-hydroxycoumarone (6) showed four singlets at $\delta$ 2.70 and 7.04, 8.00, and 12.40 for acetyl, H-7, H-4 and hydroxyl protons respectively, and two doublets at $\delta$ 6.72, and 7.56 for H-3, and H-2 protons respectively, which was in agreement with the proposed linear structure of 5-acetyl-6-hydroxycoumarone (6).

In order to obtain the desired coumarones in high yields, several polar and non-polar solvents were tried. Interestingly, the reaction in benzene or toluene at reflux temperature using Dean-Stark water-separator remarkably enhanced the yield of 5 and 6 without forming any polymerized compounds. Thus, a minor change in reaction conditions, led to yield desired coumarones, 5-acetyl-4-hydroxycoumarone (5) and 5-acetyl-6-hydroxycoumarone (6) in 38% and 54% yields, respectively. Prolonged heating of reaction mixture increased the formation of side products. To compare the efficiency of an A-15 resin with other acids, an independent reaction with PPA using Dean-Stark apparatus was carried out. Unfortunately no remarkable increase in the yield of the products 5 and 6 was noticed. Thus, the use of A-15 resin offers several advantages such as easy work-up of the reaction mixture by filtering the resin, reusability of resin and recovery of solvent.

Among the various literature procedures known for the synthesis of flavone ring, the Baker-Venkataraman rearrangement followed by acid catalyzed cyclization is the most common. The coumarone, 5-acetyl-4-hydroxycoumarone (5) was treated with aroyl chloride in pyridine to form the corresponding benzoates followed by base-catalyzed rearrangement to yield corresponding $\beta$-hydroxyfuranochalcones 9a-d in 90-94 % yield. The synthesis of the natural products, lanceolatin B (10a) and isopongaglabol methyl ether (10b) was achieved by acid-catalyzed cyclization of 9a or 9b to their corresponding angular furanoflavones in 92% and 94% yields, respectively.

![Diagram](image1.png)

The $^1$H NMR spectrum of isopongaglabol methyl ether (10b) showed two doublets at $\delta$ 7.54 and 8.15 for H-6 and H-5 protons respectively, which are specific for angular furanoflavone ring system.
Linear furanoflavones were prepared by the reaction of 5-acetyl-6-hydroxy-coumarone (6) with 3, or 4-methoxybenzoyl chloride followed by Baker-Venkataraman rearrangement to afford furanochalcones 7a-e. The compounds 7b and 7e were refluxed in a mixture of acetic acid and sulfuric acid (3:1) to yield natural products glabone (8b) and pongone (8e) in 94% and 91% yields, respectively.

The physical and the spectroscopic data of glabone did not match with the data reported by Das Kanungo et al. We resynthesized the compound 8b by preparing the intermediate 7b followed by acid-catalysed intramolecular cyclization which was found to be identical to the previously synthesized compound 8b. Another compound 3-hydroxy-1-(6-hydroxybenzofuran-5-yl)3-(3,4-methylenedioxy-phenyl)-propenone 7d was also prepared by reacting coumarone 6 with piperonyloyl chloride and its structure was confirmed by X-ray crystallography (Figure 4).

![Figure 4: X-ray crystal structure of 7d with 30% probability.](image)

Finally, we concluded that the structure initially assigned for glabone by Das Kanungo et al. was wrong. The $^1$H NMR spectrum of compound 8b showed two singlets at δ
7.44 and 8.49 ppm for H-8 and H-5 protons respectively while for compound 8e, they appeared at δ 7.69 and 8.49 ppm respectively.

On comparing the 1H NMR data of angular (10b) and linear (8b) furanoflavones, a marked difference (~0.3 ppm) in chemical shifts for H-5 and H-3' protons was observed. In angular furanoflavones H-5 & H-3' protons appeared at around δ 8.17 & 7.22 as doublets, whereas in linear furanoflavones these protons resonated as singlet and doublet at δ 8.49 & 6.92 respectively. These two characteristic peaks may be helpful to natural product chemists in assigning the angular and linear furanoflavone ring skeletons.

![Scheme 3](image)

Scheme 3: Reagents & conditions: i) CH₃I, K₂CO₃, acetone, reflux; ii) ArCOOEt, dry benzene, KH, reflux.

Finally, the synthesis of naturally occurring furanochalcones (14a-e), such as pongamol 14a and ovalitenone 14d were carried out as shown in Scheme 3. The hydroxy group of coumarone 5 was converted to methoxy group by direct methylation with methyl iodide to yield 5-acetyl-4-methoxycoumarone 13 in 95% yields. The coumarone 13 was condensed with aromatic acid esters in presence of strong base to furanochalcones, 14a-e in 85-90% yields. The structure of one of the angular furanochalcones 14c was confirmed by single crystal x-ray analysis (Figure 5).

![Figure 5](image)

Figure 5: X-ray crystal structure of 14c with 30% probability.
The $^1$H NMR spectrum of compound 14d showed four singlets at $\delta$ 4.13, 6.05, 7.06 and 16.98 for CH$_3$CO, CH$_2$, H-9 and OH protons respectively, and doublets at $\delta$ 7.30 and 7.86 for H-6 and H-7 protons respectively. This was in confirmation with the proposed structure of 14d as 3-benzo[1,3]dioxol-5-yl-3-hydroxy-1-(4-methoxy-benzofuran-5-yl)-propenone.

Similarly, linear furanochalcones 12 was synthesized from coumarone 6. All the synthesized natural products were characterized by spectroscopic analyses and/or by direct comparison with authentic samples.

In summary, the use of cation exchange resin A-15 provided extremely simple and concise method (two steps) for the preparation of 5-acetyl-4-hydroxycoumarone (5) and 5-acetyl-6-hydroxycoumarone (6), which are crucial precursors for several naturally occurring furanoflavones and furanochalcones. Earlier, the syntheses of these furanoflavonoids have been achieved in a minimum of six steps. But, our procedure (Goel, A.; Dixit, M. Synlett. 2004, 1990-1994) reduced the total synthesis only in a four steps for angular and linear furanoflavones as well as furanochalcones. This process opens a new avenue for the synthesis of various other natural and unnatural furanoflavones and furanochalcones of pharmaceutical importance.

2.4 Synthesis of hydroxybenzofuran methyl ketones and their corresponding dimers (5, 6, 15, 16, 19a,b, 21-26)

Benzofurans, dihydrobenzofurans and their dimers in isolated or rigid conformations are key structural units found in a large number of medicinal plants. They are usually active constituents of plant extracts used in traditional remedies, and play a pivotal role in the natural defence mechanisms of their sources. A few structures representatives of
naturally occurring benzofurans \(^{23}\) are shown in Figure 6. In nature's library of benzofuran derivatives, most of these benzofuran motifs are functionalized on benzenoid ring with an adjacent hydroxy and acetyl functionality but differ in their point of attachments. For example, euparin possessed a 5-acetyl-6-hydroxybenzofuran skeleton, while ageratone possessed a 6-acetyl-5-hydroxybenzofuran ring system. Similarly, 4(6)-hydroxytremetone possessed 5-acetyl-4(6)-hydroxy-2,3-dihydrobenzofuran architecture, while viscidone possessed 6-acetyl-5-hydroxy-2,3-dihydrobenzofuran ring system. These positional isomeric core structures offer real challenges to natural product chemists in assigning the correct structure for an unknown compound. In addition, these benzofurans with an adjacent hydroxy and acetyl functionality are key precursors for the synthesis of several naturally occurring angular and linear furanoflavonoids.\(^{23,16,17}\) Therefore, a synthetic route leading to ortho-hydroxy-benzofuran methyl ketones with particular attributes would be of general interest.

![Figure 6: Examples of naturally occurring benzofurans, dihydrobenzofurans and their dimers bearing hydroxy and acetyl functionalities.](image)

Numerous synthetic methodologies are available in the literature for the construction of benzofuran ring. Several new synthetic approaches have been developed in recent year.\(^{24,25}\) The most common procedures include palladium-catalyzed coupling of substituted 2-halophenols and the appropriate alkynes, a Dotz benzannulation\(^{18}\) approach using \(\alpha,\beta\)-unsaturated chromium carbene complexes and copper-catalyzed intramolecular ring closure reactions. Unfortunately, the extension of these procedures in preparing naturally occurring benzofurans suffered from the limited use of expensive palladium catalysts, the selective
preparation of organometal complexes, the protection-deprotection of free hydroxy and acetyl functionality and/or harsh reaction conditions. Recently, Kotschy et al.\textsuperscript{26} highlighted the difficulties they encountered in the total synthesis of dehydrotremetone in which Sonogashira coupling failed even under the best conditions known today. Though these natural products (Figure 6) are structurally very simple but only the partial synthesis of some of the natural benzofurans (euparin, tremetone and dehydrotremetone) using functionalized benzofurans as precursors have been reported in multi-steps.\textsuperscript{27}

\[
\begin{align*}
\text{A} & \quad \text{B} & \quad \text{C} \\
\text{D} & \quad \text{E} & \quad \text{F}
\end{align*}
\]

\textbf{Figure 7:} Six possible isomers of hydroxybenzofuran methyl ketone (A-F)

There are only six isomeric structures possible in \textit{ortho}-hydroxybenzofuran methyl ketones (A-F) with the consideration that a hydroxy group is adjacent to an acetyl group in the benzenoid ring of the benzofuran as shown in Figure 7. Although these benzofurans A-F are structurally very simple, they have been prepared mainly through manipulation on properly functionalized benzofurans in multi-steps in moderate yields.\textsuperscript{28}

\[
\begin{align*}
\text{1} \quad \text{2f} & \quad \text{3} & \quad \text{4} & \quad \text{5} & \quad \text{6} \\
\text{Resinous substance} & \quad \text{15} & \quad \text{16}
\end{align*}
\]

\textbf{Scheme 4:} Reagents & conditions: (i) \text{BrCH}_2\text{CH(OEt)}_2, \text{DMF, K}_2\text{CO}_3, 160^\circ\text{C}; (ii) A-15, toluene, reflux, Dean-stark apparatus; (iii) A-15, toluene, reflux; (iv) A-15, toluene, reflux, without Dean-stark apparatus.
We put forward two-step synthesis of isomeric benzofurans A-E together with the A
15-catalyzed controlled dimerization of these benzofurans to bibenzofurans, which are
similar to naturally occurring bis(benzofurans) and apparently formed by a similar
mechanism.

Our strategy for the synthesis of 5-acetyl-4-hydroxybenzofuran A (5) and 5-acetyl-6-
hydroxybenzofuran B (6), is based on A-15 catalysed cyclization of phenoxy acetal (2f) that
led to two minor products, identified as coumarones 5 & 6 and major product as a resinous
mixture, shown in Scheme-2. On repeated column chromatography in combination with
preparative TLC separation, we isolated two products 15 and 16 from the resinous mixture in
low yields. The mass spectrum of the compound 16 showed molecular ion peak at m/z 352,
which was corresponding to two units of benzofuran 5 or 6 (Scheme 4).

The $^1$H NMR spectroscopic analysis of 16 revealed two methyl singlets at $\delta$ 2.53 and
2.68 and two hydroxy group singlets at $\delta$ 12.46 and 13.02, and four aromatic protons at $\delta$
6.43, 6.99, 7.58 and 7.91, which confirmed the possibility of a dimer of benzofuran 6. Two
multiplets at $\delta$ 4.75-4.80 and $\delta$ 4.92-4.96 for two and one proton respectively revealed that
one of the furan rings of the dimer is reduced under acidic conditions. These dimers can be
of two types such as 2',3'-dihydro[2,3']bibenzofuran or 2',3'-dihydro[3,2']-bibenzofuran
depending upon substituents on the benzofuran ring. A literature search on dimerization of
benzofuran revealed that a natural benzofuran kellinone has been reported to form a dimer
under acidic conditions. The presence of a peak at $\delta$ 6.46 for CH-3 in $^1$H NMR spectrum of
16 and absence of a peak at around $\delta$ 7.56 for CH-2 proton allowed us to propose a structure
as 5,5'-diacetyl-6,6'-dihydroxy-2',3'-dihydro[2,3']bibenzofuran. Finally, the structure of 16
was confirmed by a single crystal X-ray analysis. The conformation of 16 along with the
atomic numbering scheme is shown in Figure 8.
Similarly other isolated product 15 was assigned as 5,5'-diacetyl-4,4'-dihydroxy-2',3'-dihydro-[2,3']bibenzofuran. The formation of bibenzofuran from phenoxyacetal revealed that as soon as the benzofuran is formed through cyclization, it got converted into bibenzofuran by self-dimerization in aqueous acidic medium. These bibenzofurans are similar to natural dimers of ageratone or dehydrotremetone as shown in Figure 6.

Scheme 5: Reagents & conditions: i) A 15, toluene, reflux, Dean-stark apparatus; ii) A 15, toluene, reflux; (iii) methyl iodide, acetone, K$_2$CO$_3$, reflux.
The utility of this route is to prepare a series of substituted benzofurans (C-F) and their dimeric bibenzofurans in a controlled manner. These benzofurans (C-F) with adjacent hydroxy and acetyl groups have been prepared either from functionalized benzofurans\textsuperscript{28a,b} or coumarins\textsuperscript{28c} in multi-steps. Our approach for preparing these useful benzofurans followed two-step procedure as demonstrated for the compounds 15 and 16.

Table 1. A-15 catalyzed dimerization of ortho-hydroxybenzofuran methyl ketones

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Product</th>
<th>Yield (%)</th>
</tr>
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<tbody>
<tr>
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<td>\begin{center} \includegraphics[width=0.2\textwidth]{15.png} \end{center}</td>
<td>89</td>
</tr>
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<td>\begin{center} \includegraphics[width=0.2\textwidth]{6.png} \end{center}</td>
<td>\begin{center} \includegraphics[width=0.2\textwidth]{16.png} \end{center}</td>
<td>81</td>
</tr>
<tr>
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<td>92</td>
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<tr>
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<tr>
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<td>86</td>
</tr>
</tbody>
</table>

The compound 6-hydroxy-7-acetylbenzofuran 18 (or C) was prepared in 85\% yield by the reaction of 2,6-dihydroxyacetophenone and bromoacetaldehyde diethylacetal to form
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A phenoxyacetal 17, followed by A 15-catalyzed cyclization under Dean-Stark condition (Scheme 5). Similarly, 6-acetyl-5-hydroxy-benzofuran 21 (or D) and 4-acetyl-5-hydroxy-benzofuran 22 (or E) were synthesized in good yields from commercially available 2,5-dihydroxyacetophenone through a phenoxyacetal intermediate 20 as shown in Scheme 5.

Apart from naturally occurring benzofurans, natural dimers have also been isolated from Ageratum houstonianum, Ophrys porus charua, and Encelia canescens in minor quantities. The biosynthetic route of such dimers have been proposed through Diels-Alder-type reaction between two units of ageratone or dehydrotreometone, followed by allylic hydroxylation or hydroperoxidation of the initial adducts. An alternative biosynthetic approach for natural dimeric chromenes and mixed dimers has been proposed through acid-catalyzed addition of hydroxytreometone to acetyl chromene followed by isomerization.

The formation of similar type of benzofuran dimers (15 and 16) from acid-catalyzed cyclization of phenoxyacetals supported the mechanism proposed by Bohlmann et al. for the formation of mixed dimers. Recently five-membered heteroaryl-based atropisomeric ligands have been developed for asymmetric synthesis. Therefore, we prepared dimers of these benzofurans (5, 6, 18, 21, 22, & 25) in the presence of A-15 separately. The precursor 25 was prepared by the reaction of cyclohexane-1,3-dione with chloroacetaldehyde in presence of base, followed by dehydration, esterification and aromatization. The compound 5 on refluxing in toluene with A-15 selectively afforded 2',3'-dihydro-[2,3']bibenzofuran (15) in 89% yield along with some unreacted benzofuran 5. Similarly other set of compounds (6, 18, 21, 22, 25; Table 1) was treated under analogous reaction conditions to afford bibenzofurans (16, 19, 23, 24, and 26) in good yields. All the synthesized compounds were characterized by spectroscopic analyses.

With these observations, we propose a possible mechanism for the formation of bibenzofuran from ortho-hydroxybenzofuran methyl ketone in the presence of A-15 catalyst (Scheme 6). The mechanism for the dimerization, using A-15 might involve protonation of benzofuran 6 to form a carbonium ion intermediate C followed by attack of another molecule of 6 at δ-position of the intermediate C to form an intermediate bibenzofuran D. This intermediate on deprotonation affords 2',3'-dihydro-[2,3']bibenzofuran 16 as shown in Scheme 6.
Scheme 6: A possible mechanism for dimerization of 5-acetyl-6-hydroxybenzofuran (6).

A highly convenient two-step general synthesis of naturally-mimicking benzofuran derivatives adjacent pendant with hydroxy and acetyl substituents were developed. (Dixit, M.; Sharon, A.; Maulik, P. R.; Goel, A. Synlett. 2006, 1497-1502.) Amberlyst-15-catalyzed controlled dimerization of these benzofurans led to yield 2',3'-dihydro-[2,3']bibenzofurans, which are taxonomically identical to the natural dimmers. In particular, the synthesis of ortho-hydroxybenzofuran methyl ketone requires concomitant removal of azeotropic mixture during cyclization of phenoxyacetals, however bibenzofurans can directly be prepared without removal of azeotropic contents. The ease of controlled cyclization and selective dimerization in the presence of a catalyst A-15 and its reusability for several times opens new avenue to explore chemical and biological potential of these interesting molecules. Most of the synthesized chalcones, furanoflavonoids were evaluated for their PTP-1B inhibitory activity, antifungal activity, antitubercular activity and antileishmanial activity.

2.5 Biodynamic properties of nature-mimicking benzofurans and their dimer, hydroxy chalcones, furanoflavonoids, furanochalcones,

2.5.1 Protein tyrosine phosphatase inhibitory activity

Vanadate is a non-selective inhibitor of PTPs, and studies have shown that treatment with vanadate can normalize blood glucose level in diabetics. The effect of synthesized
compounds on protein tyrosine phosphatase was studied by pre-incubating 100 μM of the compounds in the reaction system for 10 min. and the residual PTPase activity determined according to the method of Goldstein et al.\textsuperscript{32} Taking sodium vanadate as a control, we evaluated PTP-1B inhibitory activity of 4-alkoxy-2-hydroxyacetophenones (2a-e), benzyolated acetophenones (3a-c), hydroxychalcones (4a-c), nature-mimicking benzofurans (5, 6, 18, 21, 22, & 25), bibenzofurans (15, 16, 19a,b, 23, 24, 26), furanoflavonoids (7a-e, 8a-e, 9a-d, 10a,b) and furanochalcones (12 and 14a-e) at 100 μM concentration and their results are summarized in Table 2.

Table 2 \textit{In vitro} PTP-1B enzyme inhibitory activity for the compounds (2a-f, 3a-c, 4a-c, 5, 6, 7a-e, 8a-e, 9a-d, 10a,b, 12, 14a-c, 15-16, 19a,b, 23 & 26)

<table>
<thead>
<tr>
<th>Compound</th>
<th>% inhibition</th>
<th>Compound</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>22.4</td>
<td>8a</td>
<td>67.5</td>
</tr>
<tr>
<td>2b</td>
<td>Ni</td>
<td>8b</td>
<td>75.6</td>
</tr>
<tr>
<td>2c</td>
<td>38.6</td>
<td>8c</td>
<td>24.3</td>
</tr>
<tr>
<td>2d</td>
<td>Ni</td>
<td>8d</td>
<td>8.1</td>
</tr>
<tr>
<td>2e</td>
<td>22.4</td>
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<td>Ni</td>
</tr>
<tr>
<td>2f</td>
<td>Ni</td>
<td>9a</td>
<td>40.5</td>
</tr>
<tr>
<td>3a</td>
<td>12.8</td>
<td>9b</td>
<td>24.3</td>
</tr>
<tr>
<td>3b</td>
<td>Ni</td>
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</tr>
<tr>
<td>3c</td>
<td>Ni</td>
<td>10a</td>
<td>21.0</td>
</tr>
<tr>
<td>4a</td>
<td>Ni</td>
<td>10b</td>
<td>22.0</td>
</tr>
<tr>
<td>4b</td>
<td>Ni</td>
<td>12</td>
<td>41.5</td>
</tr>
<tr>
<td>4c</td>
<td>Ni</td>
<td>14a</td>
<td>27.0</td>
</tr>
<tr>
<td>5</td>
<td>19.0</td>
<td>14b</td>
<td>Ni</td>
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<tr>
<td>6</td>
<td>21.6</td>
<td>14c</td>
<td>42.8</td>
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<td>Ni</td>
<td>23</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26</td>
<td>55.5</td>
</tr>
</tbody>
</table>

\textit{Sodium Vanadate} (control)

\textsuperscript{4}Values are mean from three independent sets of experiments tested at 100 μM concentration; Ni means no inhibition.

The structure-activity relationship of the screened 4-alkoxy-2-hydroxyacetophenones (2a-e) revealed that bulky non-polar moiety at the terminus of alkyl group possesses good inhibitory activity (38.6%). None of the benzyolated acetophenones (3a-c) and their corresponding chalcones (4a-c) showed inhibition except 3a, which showed little activity. Rests of the compounds were either inactive or possessed low range activity.

The PTP-1B inhibitory activity of nature-mimicking benzofurans (5, 6, 18, 21, 22, 25), furanoflavonoids (7a-e, 8a-e, 9a-c, 10a,b) and furanochalcones (12 and 14a-e) at 100 μM concentration and their results are summarized in Table 2. It is evident from the activity profile of linear (10a,b) and angular (12a, b) furanoflavonoid that linear furanoflavonoids showed better inhibition (67.5, 75.6%) against PTP-1B compared to their angular isomers.
None of the intermediate benzofuran chalcones (9a-c and 7a-e) showed good inhibition.

Various hydroxy benzofuran methyl ketones and their nature-mimicking dimers have been evaluated as PTP-1B inhibitors. The structure-activity relationship of the screened benzofuran derivatives revealed that dimers (15, 16, 19a,b, 23, 26) of hydroxy-acetylbenzofurans (5, 6, 18, 21, and 25) showed good PTP-1B inhibitory activity (54.6-74.9%) except 23 compared to their monomers (19-21.6).

Recent studies have demonstrated that the formation of aggregates of nonspecific inhibitors (promiscuous inhibitors) sometimes play a major role in displaying enzyme inhibitory activity rather than a single 1:1 ligand-protein interaction. These aggregates of ~30-400 nm in diameter have shown inhibition by interacting with protein through adsorption or absorption mechanism. It has also been demonstrated that promiscuous inhibition can be prevented and reversed using an appropriate concentration of nonionic detergents such as Triton X-100, saponin, or digitonin without compromising the enzyme assay performance. In order to ruled out the possibility of promiscuous inhibition in our screening results, we re-examined the selected compounds 15, 16, 8a, 8b, 19a, 19b, 23, and 26 at 10, 25, 50, 75, and 100 μM concentration in the absence or in the presence of a detergent 0.01% Triton X-100. The IC₅₀ and the dissociation constant Kᵢ of the compounds are shown in Table 3, which suggests that the inhibitory activity of these compounds did not significantly changed by the addition of an appropriate concentration of Triton X-100, as expected for promiscuous inhibitors.

Table 3: *In vitro* PTP-1B enzyme inhibitory activity for the compounds.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>PTP-1B Inhibitory Activity</th>
<th>- Triton X-100</th>
<th>+ Triton X-100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ (μM)</td>
<td>Kᵢ (μM)</td>
<td>IC₅₀ (μM)</td>
</tr>
<tr>
<td>8a</td>
<td>94.0</td>
<td>57.0</td>
<td>61.3</td>
</tr>
<tr>
<td>8b</td>
<td>72.5</td>
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</tr>
<tr>
<td>16</td>
<td>87.0</td>
<td>35.0</td>
<td>58.8</td>
</tr>
<tr>
<td>19a</td>
<td>75.0</td>
<td>64.0</td>
<td>56.3</td>
</tr>
</tbody>
</table>

*Compounds were examined without Triton X-100; *c*omponents were examined in the presence of 0.01% Triton X-100.

In contrast, these compounds 15, 8a, 8b, 19a showed increased catalytic enzyme activity (reduced IC₅₀ values) in the presence of Triton X-100 probably due to the detergent causing a reduction in nonspecific protein binding onto the experimental plates. A decrease in enzyme inhibitory activity (or increased IC₅₀ value) was observed for the compound 16 in the presence of Triton X-100, which suggests that the inhibitory activity may be due to the
partial formation of aggregates of 16. Among all the screened compounds, the benzofuran dimers 15, 19a and linear furanoflavonoids 8a,b showed IC50 in the range of 56-69 μM with Ki of 27-54 μM.

2.5.2 In vitro antifungal activity

The several of the synthesized compound were evaluated for antifungal activity against Candida albicans, Cryptococcus neoformans, Sporothrix schenckii, Trichophyton mentagrophytes, and Candida parapsilosis (ATCC-22019), but none of various hydroxy chlacone, furanoflavonoids, furanochlacones, hydroxybenzofuran methylketones, and their dimer generate any significant results.36

2.5.3 MABA anti-tubercular screening

These compounds were also tested for the MABA anti-tubercular activity but none of the compound found active.

2.5.4 In vitro antileishmanial activity37

The compounds were tested against extracellular promastigotes of L. donavani residing within murine morphages. These compounds have exhibited inhibition of parasites in a concentration ranging from 50 to 10 mg/ml (Table 4)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Concentration (μg/mL)</th>
<th>Promastigotes(^a) (% inhibition)</th>
<th>Entry</th>
<th>Concentration (μg/mL)</th>
<th>Promastigotes(^a) (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
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<td>40.4</td>
<td>9d</td>
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</tr>
<tr>
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<td>Pentamidine</td>
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<td>98</td>
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</table>
In conclusion, we have expeditiously synthesized various nature-mimicking hydroxybenzofuran methyl ketones and their dimer as well as linear and angular furanochalcones and flavones and evaluated their biodynamic properties. The protein tyrosine phosphates-1B inhibitory activity of these nature-mimicking benzofuran dimers 15, 19a showed good inhibitory activity (IC$_{50}$: 58.8 and 56.3 µM) against PTP-1B compared to their monomers. The linear furanoflavonoids 8a,b showed better inhibition (67.5, 75.6%) against PTP-1B compared to their angular isomers 10a,b (21, 22%).

2.6 Experimental

All the chemical and reagents used were of analytical grade and were purchased from Sigma Aldrich chemical Co. $^1$H NMR and $^{13}$C NMR spectra were taken on a Bruker WM-200, Bruker WM-300, at 200 and 300MHz respectively. CDCl$_3$ was taken as the solvent. Chemical shift are reported in parts per million shift (δ-value) from Me$_4$Si (δ 0 ppm for 1H) or based on the middle peak of the solvent (CDCl$_3$) (δ 77.00 ppm for $^{13}$C NMR) as an internal standard. Signal pattern are indicate as s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Coupling constant (J) are given in hertz. Infrared (IR) spectra were recorded on a Perkin-Elmer AX-1 spectrophotometer in KBr disc and reported in wave number (cm$^{-1}$). Fast-atomic bombardment (FAB) spectrometer was used for mass spectra analysis. Melting points were measured with Buchi-530 melting point apparatus. All the reactions were carried out under anhydrous conditions and were monitored by TLC; visualization was done with UV-light (254 nm).

**General Procedure for the synthesis of compounds 2, 3 and 4**

A mixture of 2,4-dihydroxy-acetophenone (1, 6 mmol, 912 mg) with alkyl halide (6 mmol) and potassium carbonate (2 eq) was refluxed in acetone for 8-10 hrs. The resulting mixture was filtered to remove the potassium carbonate. The filtrate was concentrated to dryness to get the pure compound (2) in about 60-70 % yield. An acetophenone derivatives (2, 6 mmol) and aryl chloride (6 mmol) in dry pyridine (5 mL) was stirred at room temperature for an hour before heating at 100 °C for 10 min. under anhydrous condition. The resulting solution was poured into 1M HCl containing crushed ice, and filtered the precipitate, yielded solid benzoate (3) in about 85% yield. The potassium tertiary butoxide was slowly added to magnetically stirred solution of the benzoate (3) and reaction mixture
was further stirred for about 5 hrs in dry pyridine. The resulting solution mixture was added to 10% acetic acid solution. The yellow solid obtained was filtered, dried and crystallized in hexane-ethyl acetate to give yellow needle like crystals in good yield as 4.

\[4\text{-Hydroxy-3\-(3\text{-hydroxy-3-phenyl-acryloyl)-phenoxy}}\text{acetonitrile (4a)}\]
Yield: 73%; MP: 119-120 °C; MS (FAB): m/z 296 (M^+1); IR (KBr) 1609 (CO), 2368 (CN), 3428 cm^{-1} (OH); ^1H NMR (200 MHz, CDCl3) δ 4.81 (s, 2H, CH₂), 6.53-6.59 (m, 2H, ArH), 6.74 (s, 1H, CH), 7.49-7.57 (m, 3H, ArH), 7.78 (d, J = 8.1 Hz, ArH), 7.93 (d, J = 6.2 Hz, ArH), 12.56 (s, 1H, OH), 15.36 (s, 1H, OH).

\[4\text{-[3\-(4\text{-Fluoro-phenyl)-3-hydroxy-acryloyl}-3\text{-hydroxy-phenoxy}}\text{acetonitrile (4b)}\]
Yield: 63%; m.p: 129-130°C; MS (FAB): m/z 314 (M^+1); IR (KBr) 1608 (CO), 2368 (CN), 3441 cm^{-1} (OH); ^1H NMR (200 MHz, CDCl3) δ 4.81 (s, 2H, CH₂), 6.53-6.58 (m, 2H, ArH), 6.67(s, 1H, CH); 7.13-7.22 (m, 2H, ArH); 7.75 (d, J = 8.6 Hz, 1H, ArH); 7.91-7.98 (m, 2H, ArH); 12.48 (s, 1H, OH); 15.41 (s, 1H, OH).

\[3\text{-[4\text{-Fluoro-phenyl)-3-hydroxy-1-\-(2\text{-hydroxy-4-phenethyloxy-phenyl})-propenone (4c)}\]
Yield: 71%; m.p: 98-99 °C; MS (FAB): m/z 379 (M^+1); IR (KBr) 1629 (CO), 3457 cm^{-1} (OH); ^1H NMR (200 MHz, CDCl3) δ 3.11 (t, 2H, J = 7.0 Hz, CH₂), 4.22 (t, 2H, J = 7.0 Hz, CH₂), 6.49-6.40 (m, 2H, ArH), 6.65 (s, 1H, CH), 7.20-7.11 (m, 2H, ArH), 7.32-7.25 (m, 5H, ArH), 7.65-7.62 (m, 2H, ArH), 7.96-7.88 (m, 2H, ArH), 12.47 (s, 1H, OH), 15.42 (s, 1H, OH).

\[3\text{-[4\text{-Fluoro-phenyl)-3-hydroxy-1-\-(2\text{-hydroxy-4-phenethyloxy-phenyl})-propenone (4d)}\]
Yield: 52%; m.p: 120-121° C; MS (FAB): m/z 361 (M^+1); IR (KBr) 1608 (CO), 3454 cm^{-1} (OH); ^1H NMR (200 MHz, CDCl3) δ 3.11 (t, 2H, J = 7.0 Hz, CH₂), 4.23 (t, 2H, J = 7.0 Hz, CH₂), 6.45-6.49 (m, 2H, ArH), 6.71 (s, 1H, CH), 7.26-7.33 (m, 4H, ArH), 7.45-7.52 (m, 4H, ArH), 7.68 (d, 1H, J = 9.0 Hz, ArH), 7.88-7.94 (m, 2H, ArH), 12.54 (s, 1H, OH), 15.36 (s, 1H, OH).

\[1\text{-[4\text{-Allyloxy-2-hydroxy-phenyl)-3\-(4\text{-fluoro-phenyl)-3-hydroxy-propenone (4f)}\]
Yield: 65%; m.p: 122-124° C; MS (FAB): 315 (M^+1); IR (KBr) 1633 (CO); 3457 cm^{-1} (OH); ^1H NMR (200 MHz, CDCl3) δ 4.58 (d, 2H, J = 4.48 Hz, OCH₂); 5.48-5.30 (m, 2H, CH₂); 5.96-6.0 (m, 1H, CH); 6.53-6.46 (m, 2H, ArH); 6.66 (s, 1H, CH); 7.11-7.20 (m, 2H,
ArH); 7.68 (d, 1H, J = 8.6 Hz, ArH); 7.89-7.96 (m, 2H, ArH), 12.50 (s, 1H, OH); 15.42 (s, 1H, OH).

**General procedure for synthesis of hydroxy benzofuran methyl ketone (5, 6, 18, 21, and 22)**

A mixture of dihydroxyacetophenone (9.12 gms, 60 mmol) and bromoacetaldehyde diethylacetal (90 mmol) in dry DMF using K2CO3 as base was stirred at 135°C for 30 hrs. The resulting mixture was neutralized with 1N HCl and extracted with EtOAc, the organic layer was separated, dried over Na2SO4 and concentrated under reduce pressure to get the crude material. The compounds (2f, 17, and 20) were purified on silica gel column using EtOAc-Hexane (1:10) as eluent, in 70-80% yield.

The phenoxyacetals 2f, 17, or 20 (20 g, 0.075 mol) in dry toluene (30 mL) was refluxed with Amberlyst 15 (2.5 g) at 120 °C using Dean–Stark water separator for 10 hrs. The resulting reaction mixture was filtered and the resin was washed with excess of toluene. The filtrate thus obtained was concentrated to dryness and pure compounds (5, 6, 18, 21, and 22) were isolated by silica gel column chromatography using EtOAc–hexane (1:10) as eluent, in 92-97% yield.

**1-(4-Hydroxy-benzofuran-5-yl)-ethanone (5)**

White solid; yield 38%; m.p: 92-93°C (lit.12 92-93°C); MS (FAB): m/z 177 (M+1); IR (KBr): 1640 (CO), 3421 cm\(^{-1}\) (OH); \(^1\)H NMR (200 MHz, CDCl3): \(\delta\) 2.66 (s, 3H, CH3), 7.00 (d, J = 2.2 Hz, 1H, H-3), 7.04 (d, 1H, J = 8.8 Hz, H-7), 7.57 (d, 1H, J = 2.2 Hz, H-2), 7.66 (d, 1H, J = 8.8 Hz, H-6), 13.28 (s, 1H, OH).

**1-(6-Hydroxy-benzofuran-5-yl)-ethanone (6)**

Yellow solid; yield 54%; mp: 101–102°C (lit.10 96°C); MS (FAB): m/z 177 (M+ 1); IR (KBr): 1638 (CO), 3426 (OH) cm\(^{-1}\). \(^1\)H NMR (200 MHz, CDCl3): \(\delta\) 2.70 (s, 3H, CH3), 6.72 (d, 1H, J = 2.2 Hz, H-3), 7.04 (d, 1H, J = 8.8 Hz, H-7), 7.56 (d, 1H, J = 2.2 Hz, H-2), 8.00 (s, 1H, H-4), 12.40 (s, 1H, OH).

**1-(6-Hydroxy-benzofuran-7-yl)-ethanone (18)**

White solid; yield 96%; m.p: 111-112°C (lit.22c 110°C); MS (FAB): m/z 177 (M+1); IR (KBr): 1638 (CO), 3432 cm\(^{-1}\) (OH); \(^1\)H NMR (200 MHz, CDCl3): \(\delta\) 2.90 (s, 3H, CH3), 6.76 (d, J = 2.2 Hz, 1H, H-3), 6.90 (d, 1H, J = 8.6 Hz, H-5), 7.62 (d, 1H, J = 2.2 Hz, H-2), 7.66 (d, 1H, J = 8.6 Hz, H-4), 12.85 (s, 1H, OH).
1-(5-Hydroxy-benzofuran-6-yl)-ethanone (21)
White solid; yield 56%; m.p: 120-121°C; MS (FAB): m/z 177 (M⁺+1); IR (KBr): 1629 (CO), 3447 cm⁻¹ (OH); ¹H NMR (200 MHz, CDCl₃): δ 2.60 (s, 3H, CH₃), 6.86 (s, 1H, ArH), 6.91 (d, J = 2.2 Hz, 1H, H-3), 7.02 (s, 1H, ArH), 7.19 (d, 1H, J = 2.2 Hz, H-2), 11.81 (s, 1H, OH).

1-(5-Hydroxy-benzofuran-4-yl)-ethanone (22)
White solid; yield 35%; m.p: 98-99°C (lit. 22 103-104°C); MS (FAB): m/z 177 (M⁺+1); IR (KBr): 1628 (CO), 3443 cm⁻¹ (OH); ¹H NMR (200 MHz, CDCl₃): δ 2.80 (s, 3H, CH₃), 6.94 (d, 1H, J = 9.0 Hz, CH), 6.98 (d, 1H, J = 2.2 Hz, H-3), 7.64 (d, 1H, J = 9.0 Hz, ArH), 7.76 (d, 1H, J = 2.2 Hz, H-2), 13.02 (s, 1H, OH).

1-(5-Hydroxy-benzofuran-4-yl)-ethanone (25)
Following the procedure of Lee et. al.,¹⁷; white solid; yield 55%; m.p: 104-105°C (lit.¹⁷ 105°C); MS (FAB): m/z 193 (M⁺+1); IR (KBr): 1678 (CO), 3502 cm⁻¹ (OH); ¹H NMR (200 MHz, CDCl₃): δ 3.97 (s, 3H, OCH₃), 6.98 (d, 1H, J = 2.2 Hz, H-7), 7.57 (d, 1H, J = 2.2 Hz, H-2), 7.78 (d, 1H, J = 8.8 Hz, H-6), 13.18 (s, 1H, OH).

General Procedure for the synthesis of compounds (7a-e and 9a-c):
The mixture of 5 or 6 (6 mmol) and aroyl chloride (6 mmol) in dry pyridine (5 mL) was stirred at room temperature for an hour before heating at 100°C for 10 min. under anhydrous condition. The resulting solution was poured into 1M HCl containing crushed ice, which yielded a solid benzoate in about 85% yield. The potassium tertiary butoxide was slowly added to magnetically stirred solution of the benzoate for about 5 hrs in dry pyridine. The resulting solution mixture was added to 10% acetic acid solution. The yellow solid of 7a-e or 9a-b thus obtained was filtered, dried and crystallized in hexane-ethyl acetate to give yellow needle like crystals in good yield.

3-Hydroxy-1-(6-hydroxy-benzofuran-5-yl)-3-phenyl-propenone (7a)
Yellow solid; Yield: 85%; m.p: 125-126°C; MS (FAB): m/z 281 (M⁺+1); IR (KBr): 1606 (CO) cm⁻¹; ¹H NMR (200 MHz, CDCl₃): δ 6.73 (d, 1H, J = 2.2 Hz, CH), 6.89 (s, 1H, CH), 7.07 (s, 1H, ArH), 7.45-7.61 (m, 4H, CH & ArH), 7.95-7.99 (m, 2H, ArH), 8.05 (s, 1H, ArH), 12.24 (s, 1H, OH), 15.43 (s, 1H, OH); HRMS calcd. for C₁₇H₁₂O₄ 280.0736, Found 280.0694.

3-Hydroxy-1-(6-hydroxy-benzofuran-5-yl)-3-(4-methoxy-phenyl)-propenone (7b)
Yield: 88%; m.p: 155–156°C; MS (FAB): m/z 311 (M^+1); IR (KBr): 1606 cm⁻¹ (CO); \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\) 3.90 (s, 3H, OCH\(_3\)), 6.74 (d, 1H, \(J = 2.2\) Hz, CH), 6.82 (s, 1H, CH), 7.01 (d, 2H, \(J = 9.2\) Hz, ArH), 7.07 (s, 1H, ArH), 7.55 (d, 1H, \(J = 2.2\) Hz, CH), 7.94 (d, 2H, \(J = 9.2\) Hz, ArH), 8.04 (s, 1H, ArH), 12.30 (s, 1H, OH), 15.64 (s, 1H, OH); HRMS calcd. for C\(_{18}\)H\(_{14}\)O\(_3\) 310.0841, Found 310.0827.

3-(3,4-Dimethoxy-phenyl)-3-hydroxy-1-(6-hydroxy-benzofuran-5-yl)-propenone (7c)
Yellow solid; Yield: 91%; m.p: 138-140°C, MS (FAB): m/z 341 (M^+1); IR (KBr) 1611 (CO), 3418 (OH) cm⁻¹; \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\) 3.97 (s, 3H, OCH\(_3\)), 4.00 (s, 3H, OCH\(_3\)), 6.74 (d, 1H, \(J = 2.2\) Hz, CH), 6.81 (s, 1H, CH), 6.95 (d, \(J = 8.4\) Hz, ArH), 7.08 (s, 1H, ArH), 7.46-7.64 (m, 3H, CH & ArH), 8.03 (s, 1H, ArH), 12.30 (s, 1H, OH), 15.64 (s, 1H, OH).

3-Benzo[1,3]dioxol-5-yl-3-hydroxy-1-(6-hydroxy-benzofuran-5-yl)-propenone (7d)
Yellow solid; Yield: 88%; m.p: 140-142°C; MS (FAB): m/z 325 (M^+1); IR (KBr) 1626 (CO), 3424 (OH) cm⁻¹; \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\) 6.10 (s, 2H, CH\(_2\)), 6.76 (d, 1H, \(J = 2.2\) Hz, CH), 6.79 (s, 1H, CH), 6.93 (d, \(J = 8.1\) Hz, 1H, ArH), 7.09 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.54-7.62 (m, 2H, CH & ArH), 8.05 (s, 1H, ArH), 12.28 (s, 1H, OH), 15.65 (s, 1H, OH).

3-Hydroxy-1-(6-hydroxy-benzofuran-5-yl)-3-(3-methoxy-phenyl)-propenone (7e)
Yellow solid; Yield: 92%; m.p: 108–109°C; MS (FAB): m/z 311 (M^+1); IR (KBr) 1608 cm⁻¹ (CO); \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\) 3.90 (s, 3H, OCH\(_3\)), 6.74 (d, 1H, \(J = 2.2\) Hz, CH), 6.87 (s, 1H, CH), 7.08-7.12 (m, 2H, ArH), 7.36-7.52 (m, 3H, ArH), 7.56 (d, 1H, \(J = 2.2\) Hz, CH), 8.05 (s, 1H, ArH), 12.24 (s, 1H, OH), 15.43 (s, 1H, OH).

3-Hydroxy-1-(4-hydroxy-benzofuran-5-yl)-3-phenyl-propenone (9a)
Yellow solid, Yield: 88%; m.p: 150–151°C (lit\(^9\) 146°C); MS (FAB): m/z 281 (M^+1); IR (KBr): 1606 cm⁻¹ (CO); \(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\) 6.83 (s, 1H, CH), 7.01 (d, 1H, \(J = 2.2\) Hz, CH), 7.07 (d, 1H, \(J = 8.8\) Hz, ArH), 7.49-7.54 (m, 3H, ArH), 7.58 (d, 1H, \(J = 2.2\) Hz, CH), 7.73 (d, 1H, \(J = 8.8\) Hz, ArH), 7.95 (d, 2H, \(J = 8.1\) Hz, ArH), 13.05 (s, 1H, OH), 15.45 (s, 1H, OH); HRMS calcd. for C\(_{17}\)H\(_{12}\)O\(_4\) 280.0736, Found 280.0766.

3-Hydroxy-1-(4-hydroxy-benzofuran-5-yl)-3-(4-methoxy-phenyl)-propenone (9b)

76
Yield: 90%; m.p: 129–130°C; MS (FAB): m/z 311 (M^+1); IR (KBr) 1616 cm^{-1} (CO); 1H NMR (200 MHz, CDCl₃) δ 3.89 (s, 3H, OCH₃), 6.76 (s, 1H, CH), 6.94–7.02 (m, 3H, ArH), 7.08 (d, 1H, J = 8.8 Hz, ArH), 7.58 (d, 1H, J = 2.2 Hz, CH), 7.71 (d, 1H, J = 8.8 Hz, ArH), 7.93 (d, 2H, J = 9.0 Hz, ArH), 13.01 (s, 1H, OH), 15.66 (s, 1H, OH); HRMS calcd. for C₁₈H₁₄O₅ 310.0841, Found 310.0825.

3-Hydroxy-1-(4-hydroxy-benzofuran-5-yl)-3-(4-trifluoromethyl-phenyl)-propenone(9c)

Yield: 85%; m.p: 150–151°C; MS (FAB): m/z 349 (M^+1); IR (KBr) 1618 cm^{-1} (CO); 1H NMR (200 MHz, CDCl₃) δ 6.83 (s, 1H, CH), 7.02 (d, 1H, J = 2.2 Hz, CH), 7.10 (d, 1H, J = 8.9 Hz, ArH), 7.60 (d, 1H, J = 2.2 Hz, CH), 7.75 (d, 2H, J = 7.3 Hz, ArH), 7.71 (d, 1H, J = 8.8 Hz, ArH), 8.05 (d, 2H, J = 8.2 Hz, ArH), 112.93 (s, 1H, OH), 15.33 (s, 1H, OH);

General Procedure for the synthesis of compounds (8a-e and 10a-b):

The mixture of 7 or 9 (6 mmol) and H₂SO₄-CH₃COOH (1:3) was refluxed at water bath for 1 h and after that it was poured in crushed ice to afford the precipitate. The precipitate thus obtained is purified by silica-gel column using hexane-ethylacetate as eluent to get compound 8a-e or 10a-b.

7-Phenyl-furo[3,2-g]chromen-5-one (8a)

White solid, Yield 90%; m.p: 127–128°C; MS (FAB): m/z 263 (M^+1); IR (KBr): 1628 (CO) cm^{-1}; ¹H NMR (200 MHz, CDCl₃): δ 6.83 (s, 1H, ArH), 6.93 (d, 1H, J = 2.2 Hz, CH), 7.52–7.58 (m, 3H, ArH), 7.69 (s, 1H, ArH), 7.75 (d, 1H, J = 2.2 Hz, CH), 7.94–8.00 (m, 2H, ArH), 8.50 (s, 1H, ArH); HRMS calcd. for C₁₇H₁₀O₃ 262.0630, Found 262.0629.

7-(4-Methoxy-phenyl)-furo[3,2-g]chromen-5-one (8b)

White solid, Yield: 94%; m.p: 238-239°C; MS (FAB): m/z 293 (M^+1); IR (KBr): 1630 cm^{-1} (CO); ¹H NMR (200 MHz, CDCl₃): δ 3.91 (s, 3H, OCH₃), 6.76 (s, 1H, ArH), 6.93 (d, 1H, J = 2.2 Hz, CH), 7.05 (d, 2H, J = 8.8 Hz, ArH), 7.44 (s, 1H, ArH), 7.67 (d, 1H, J = 2.2 Hz, CH), 7.92 (d, 2H, J = 8.8 Hz, ArH), 8.49 (s, 1H, ArH).

7-(3,4-Dimethoxy-phenyl)-furo[3,2-g]chromen-5-one (8c)

White solid, Yield 94%; m.p: 174-175°C; MS (FAB): m/z 323 (M^+1); IR (KBr): 1628 (CO) cm^{-1}; ¹H NMR (200 MHz, CDCl₃): δ 3.98 (s, 3H, OCH₃), 4.01 (s, 3H, OCH₃), 6.77 (s, 1H,
ArH), 6.93 (d, 1H, J = 2.2 Hz, ArH), 7.01 (d, 1H, J = 8.4 Hz, ArH), 7.43 (s, 1H, ArH), 7.60 (d, 1H, J = 8.4 Hz, ArH), 7.69 (s, 1H, ArH), 7.75 (d, 1H, J = 2.2 Hz, CH), 8.49 (s, 1H, ArH).

7-Benzof[1,3]dioxol-5-yl-furo[3,2-g]chromen-5-one (8d)
White solid, Yield: 93%; m.p: 255-256°C; MS (FAB): m/z 307 (M+1); IR (KBr): 1625 cm⁻¹ (CO); ¹H NMR (200 MHz, CDCl₃): δ 6.09 (s, 2H, CH₂), 6.71 (s, 1H, ArH), 6.90-6.99 (m, 2H, CH & ArH), 7.41 (s, 1H, ArH), 7.54 (d, 2H, J = 8.10 Hz, ArH), 7.66 (s, 1H, ArH), 7.74 (d, 1H, J = 2.2 Hz, CH), 8.48 (s, 1H, ArH).

7-(3-Methoxy-phenyl)-furo[3,2-g]chromen-5-one (8e)
White solid, Yield: 91%; m.p: 188-189°C (lit. 5d 187-188 °C); MS (FAB): m/z 293 (M+1); IR (KBr): 1635 cm⁻¹ (CO); ¹H NMR (200 MHz, CDCl₃): δ 3.91 (s, 3H, OCH₃), 6.82 (s, 1H, ArH), 6.92 (d, 1H, J = 2.2 Hz, CH), 7.09 (d, 1H, J = 8.2 Hz, ArH), 7.41-7.57 (m, 3H, ArH), 7.69 (s, 1H, ArH), 7.75 (d, 1H, J = 2.2 Hz, CH), 8.49 (s, 1H, ArH).

2-Phenyl-furo[2,3-h]chromen-4-one (10a)
White solid, Yield: 92%; m.p: 127-128°C (lit. 3d 127 °C); MS (FAB): m/z 263 (M+1); IR (KBr) 1646 cm⁻¹ (CO); ¹H NMR (200 MHz, CDCl₃): δ 6.90 (s, 1H, ArH), 7.23 (d, 1H, J = 2.2 Hz, CH), 7.56-7.60 (m, 4H, ArH), 7.78 (d, 1H, J = 2.2 Hz, CH), 7.96-8.00 (m, 2H, ArH), 8.18 (d, 1H, J = 8.8 Hz, ArH).

2-(4-Methoxy-phenyl)-furo[2,3-h]chromen-4-one (10b)
White solid, Yield: 94%; m.p: 218-219°C (lit. 3f 218-219 °C); MS (FAB): m/z 293 (M+1); IR (KBr) 1655 cm⁻¹ (CO); ¹H NMR (200 MHz, CDCl₃): δ 3.90 (s, 3H, OCH₃), 6.81 (s, 1H, ArH), 7.06 (d, 2H, J = 8.8 Hz, ArH), 7.21 (d, 1H, J = 2.2 Hz, ArH), 7.58 (d, 1H, J = 8.8 Hz, ArH), 7.78 (d, 1H, J = 2.2 Hz, CH), 7.94 (d, 2H, J = 8.8 Hz, ArH), 8.17 (d, 1H, J = 8.8 Hz, ArH).

General Procedure for the synthesis of compounds (12 and 14a-c):
The mixture of benzofuran 5 or 6 (1 mmol), K₂CO₃ (1.5 mmol), methyl iodide (1.5 mol) in acetone was refluxed for 7-8h and reaction mixture was filtered to get crude, which was subjected to column chromatography to get the compound 11 or 13 in good yield. The compound 11 or 13 (1 mmol), was condensed with aryl ester (1.2 mmol), using potassium hydride (1.5 mmol, 60% in mineral oil) as base and dry benzene (7 ml) as solvent for 4-5 h, the reaction mixture was neutralized with 10 % CH₃COOH and crude thus obtained was
column chromatography using silica gel having 3% ethyl acetate in Hexane to get the compound 12 or 14 in good isolated yield.

3-Hydroxy-1-(6-methoxy-benzofuran-5-yl)-3-phenyl-propenone (12)
Yellow solid, Yield 85%, m.p: 116-118°C; MS (FAB): m/z 295 (M+ +1); IR (KBr) 1593 cm⁻¹ (CO); ¹H NMR (300 MHz, CDCl₃): δ 4.0 (s, 3H, OCH₃), 6.77 (d, 1H, J = 2.2 Hz, CH), 7.12 (s, 1H, CH), 7.15 (s, 1H, ArH), 7.45-7.53 (m, 3H, ArH), 7.58 (d, 1H, J = 2.2 Hz, CH), 7.92-8.01 (m, 2H, ArH), 8.19 (s, 1H, ArH).

3-Hydroxy-1-(4-methoxy-benzofuran-5-yl)-3-(4-methoxy-phenyl)-propenone (14a)
Yellow solid, Yield 81%, m.p: 84-86°C; MS (FAB): m/z 325 (M+ +1); IR (KBr) 1597 cm⁻¹ (CO); ¹H NMR (200 MHz, CDCl₃): δ 3.89 (s, 3H, OCH₃), 4.13 (s, 3H, OCH₃), 6.95-7.02 (m, 3H, CH & ArH), 7.10 (s, 1H, ArH), 7.31 (d, 1H, J = 8.7 Hz, ArH), 7.62 (d, 1H, J = 2.2 Hz, CH), 7.86 (d, 1H, J = 8.7 Hz, ArH), 7.96 (d, 2H, J = 8.8 Hz, ArH); HRMS calcd. for C₁₉H₁₆O₅ 324.0998, Found 324.0950.

3-(2-Chloro-phenyl)-3-hydroxy-1-(4-methoxy-benzofuran-5-yl)-propenone (14b)
Yellow solid, Yield 71%, m.p: 98-99°C; MS (FAB): m/z 331, 329 (M+ +1); IR (KBr) 1599 cm⁻¹ (CO); ¹H NMR (300 MHz, CDCl₃): δ 4.14 (s, 3H, OCH₃), 6.99 (d, 1H, J = 2.2 Hz, CH), 7.06 (s, 1H, ArH), 7.30 (d, 1H, J = 8.7 Hz, ArH), 7.62 (d, 1H, J = 2.2 Hz, CH), 7.68-7.72 (m, 1H, ArH), 7.90 (d, 1H, J = 8.7 Hz, ArH).

3-Benzo[1,3]dioxol-5-yl-3-hydroxy-1-(4-methoxy-benzofuran-5-yl)-propenone (14c)
Yellow solid; Yield 87%; m.p: 118-119°C; MS (FAB): m/z 339 (M+ +1); IR (KBr) 1655 cm⁻¹ (CO); ¹H NMR (300 MHz, CDCl₃) δ 4.13 (s, 3H, OCH₃), 6.05 (s, 2H, CH₂), 6.89 (d, 1H, J = 8.1 Hz, ArH), 6.99 (d, 1H, J = 2.2 Hz, ArH), 7.06 (s, 1H, CH), 7.30 (d, 1H, J = 8.8 Hz, ArH), 7.46 (s, 1H, ArH), 7.58 (d, 1H, J = 8.1 Hz, ArH), 7.62 (d, 1H, J = 2.2 Hz, CH), 7.86 (d, 1H, J = 8.8 Hz, CH), 16.98 (s, 1H, OH).

3-Benzo[1,3]dioxol-5-yl-3-hydroxy-1-(4-methoxy-benzofuran-5-yl)-propenone
Yellow solid, Yield 93%, mp: 158-159°C; MS (FAB): m/z 339 (M+ +1); IR (KBr) 1596 cm⁻¹ (CO); 3437 cm⁻¹ (OH); ¹H NMR (200 MHz, CDCl₃): δ 4.13 (s, 3H, OCH₃), 6.05 (s, 3H, CH₂), 6.89 (d, 1H, J = 8.1 Hz, ArH), 6.99 (d, 1H, J = 2.2 Hz, CH), 7.06 (s, 1H, ArH), 7.30 (d, 1H, J = 8.7 Hz, ArH), 7.46 (s, 1H, ArH), 7.58 (d, 1H, J = 8.7 Hz, ArH), 7.62 (d, 1H, J = 2.2 Hz, CH), 7.89 (d, 1H, J = 7.8 Hz, ArH).
3-(3,5-Dimethyl-phenyl)-3-hydroxy-1-(4-methoxy-benzofuran-5-yl)-propenone (14e)
Yellow solid, Yield 85%, mp: 130-131°C; MS (FAB): m/z 323 (M⁺ +1 ); IR (KBr) 2228 cm⁻¹ (CN); ¹H NMR (200 MHz, CDCl₃): δ 2.36 (s, 3H, CH₃), 2.53 (s, 3H, CH₃), 4.14 (s, 3H, OCH₃), 6.78 (s, 1H, H-4'), 6.98 (d, 1H, J = 2.2 Hz, H-3), 7.16 (s, 1H, H-9), 7.28 (s, 1H, H-2'), 7.30 (d, 1H, J = 8.7 Hz, H-7), 7.39 (s, 1H, H-6'), 7.62 (d, 1H, J = 2.2 Hz, H-2), 7.87 (d, 1H, J = 8.7 Hz, H-6).

General procedure for synthesis of 2',3'-dihydro-[2,3']bibenzofuran (15, 16, 19a, 23, 24, and 26)
The benzofuran (5, 6, 18, 21, 22, or 25) was refluxed in toluene (35 mL) with A-15 (30% w/w) at 120°C for 6-10 hrs. The resulting reaction mixture was filtered and the resin was washed with excess of toluene. The filtrate thus obtained was concentrated to dryness and a pure compound was isolated by silica gel column chromatography using 7% EtOAc in hexane as eluent.

1-(5-Acetyl-4,4'-dihydroxy-2',3'-dihydro-[2,3']bibenzofuranyl-5'-yl)-ethanone (15)
Yellow solid; Yield: 81%; m.p: 189-190°C; MS (FAB): m/z 353 (M⁺+1); IR (KBr): 1641 (CO), 3427 cm⁻¹ (OH); ¹H NMR (200 MHz, CDCl₃): δ 2.53 (s, 3H, CH₃), 2.68 (s, 3H, CH₃), 4.75-4.80 (m, 2H, CH₂), 4.92-4.96 (m, 1H, CH), 6.43 (s, 1H, ArH), 6.46 (s, 1H, CH), 6.99 (s, 1H, ArH), 7.58 (s, 1H, ArH), 7.91 (s, 1H, ArH), 12.46 (s, 1H, OH), 13.02 (s, 1H, OH); HRMS calcd. for C₂₄H₂₀N₂ 336.1627, Found 336.1599.

1-(5-Acetyl-6,6'-dihydroxy-2',3'-dihydro-[2,3']bibenzofuranyl-5'-yl)-ethanone (16)
Yellow solid; Yield: 89%; m.p: 149-150°C; MS (FAB): m/z 353 (M⁺+1); IR (KBr): 1638 (CO), 3433 cm⁻¹ (OH); ¹H NMR (200 MHz, CDCl₃): δ 2.57 (s, 3H, CH₃), 2.64 (s, 3H, CH₃), 4.81-4.88 (m, 1H, CH), 4.89-4.94 (m, 2H, CH₂), 6.48 (d, 1H, J = 8.6 Hz, ArH), 6.68 (s, 1H, CH), 6.96 (d, 1H, J = 8.8 Hz, ArH), 7.60 (d, 1H, J = 8.8 Hz, ArH), 7.70 (d, 1H, J = 8.6 Hz, ArH), 12.83 (s, 1H, OH), 13.15 (s, 1H, OH); HRMS calcd. for C₂₄H₂₀N₂ 336.1627, Found 336.1599.

1-(7'-Acetyl-6,6'-dihydroxy-2',3'-dihydro-[2,3']bibenzofuranyl-7'-yl)-ethanone (19a)
Chapter 2
Benzofuran Scaffold: Synthesis and Biodynamic properties

Yellow solid; Yield: 92%; m.p: 110–111°C; MS (FAB): m/z 353 (M⁺+1); IR (KBr): 1637 (CO), 3449 cm⁻¹ (OH); ¹H NMR (200 MHz, CDCl₃): δ 2.69 (s, 3H, CH₃), 2.82 (s, 3H, CH₃), 4.80-4.88 (m, 2H, CH₂), 4.97-5.02 (m, 1H, CH), 6.44 (s, 1H, CH), 6.52 (d, 1H, J = 8.4 Hz, ArH), 6.88 (d, 1H, J = 8.6 Hz, ArH), 7.30 (d, 1H, J = 8.4 Hz, ArH), 7.56 (d, 1H, J = 8.6 Hz, ArH), 12.74 (s, 1H, OH), 12.81 (s, 1H, OH); ¹³C NMR (50.32 MHz CDCl₃): δ 31.6 (CH₃), 32.0 (CH₃), 41.1 (CH), 77.2 (CH₂), 103.5 (≈CH), 107.3, 107.5, 110.3, 114.5, 117.2, 120.5, 128.5, 131.9, 154.2, 156.5, 161.8, 161.9, 164.0, 202.3, 203.5; HRMS calcd. for C₂₄H₂₀N₂ 336.1627, Found 336.1599.

1-(6'-Acetyl-5,5'-dihydroxy-2',3'-dihydro-[2,3']bibenzofuranyl-6-yl)-ethanone (23)
Yellow solid; Yield: 75%; m.p: 192–193 °C; MS (FAB): m/z 353 (M⁺+1); IR (KBr): 1648 (CO), 3422 cm⁻¹ (OH); ¹H NMR (200 MHz CDCl₃): δ 2.53 (s, 3H, CH₃), 2.68 (s, 3H, CH₃), 4.75-4.80 (m, 2H, CH₂), 4.82-4.99 (m, 1H, CH), 6.43 (s, 1H, ArH), 6.46 (s, 1H, CH), 6.99 (s, 1H, ArH), 7.58 (s, 1H, ArH), 7.91 (s, 1H, ArH), 12.47 (s, 1H, OH), 13.03 (s, 1H, OH); HRMS calcd. for C₂₄H₂₀N₂ 336.1627, Found 336.1599.

1-(4-Acetyl-5,5'-dihydroxy-2',3'-dihydro-[2,3']bibenzofuranyl-4'-yl)-ethanone (24)
Yellow semi-solid; Yield: 83%; MS (FAB): m/z 353 (M⁺+1); IR (KBr): 1645 (CO), 3437 cm⁻¹ (OH); ¹H NMR (200 MHz CDCl₃): δ 2.50 (s, 3H, CH₃), 2.57 (s, 3H, CH₃), 4.73-4.88 (m, 3H, CH & CH₂), 6.41 (d, 1H, J = 8.6 Hz, ArH), 6.61 (s, 1H, CH), 6.95 (d, 1H, J = 8.6 Hz, ArH), 7.50-7.66 (m, 2H, ArH), 12.76 (s, 1H, OH), 13.08 (s, 1H, OH); HRMS calcd. for C₂₄H₂₀N₂ 336.1627, Found 336.1599.

4,4'-Dihydroxy-2',3'-dihydro-[2,3']bibenzofuranyl-5,5'-dicarboxylic acid dimethyl ester (26)
Yellow solid; Yield: 86%; m.p: 184-185 °C; MS (FAB): m/z 385 (M⁺+1); IR (KBr): 1678 (CO), 3426 cm⁻¹ (OH); ¹H NMR (200 MHz CDCl₃): δ 3.91 (s, 3H, OCH₃), 3.95 (s, 3H, OCH₃), 3.95 (s, 3H, OCH₃), 4.78-4.95 (m, 3H, CH & CH₂), 6.65 (s, 1H, CH), 6.47 (d, 1H, J = 8.6 Hz, ArH), 6.96 (d, 1H, J = 8.8 Hz, ArH), 7.72 (d, 1H, J = 8.8 Hz, ArH), 7.81 (d, 1H, J = 8.6 Hz, ArH), 12.42 (s, 1H, OH), 13.01 (s, 1H, OH).

Procedure for the synthesis of compound 1-(5-Acetyl-6,6'-dimethoxy-2',3'-dihydro-[2,3']bibenzofuranyl-5'-yl)-ethanone (19b):

The mixture of 19a (1 mmol) with methyl iodide (1.2 mmol) in the presence of K₂CO₃ (1.5 mmol) and acetone (10 ml) was reflux for 5h and after completion of reaction;
reaction mixture was filtered and evaporated under vacuum to yield the compound 19b as colorless oil in good yield.

Yield: 95%; colorless oil; MS (FAB): m/z 380 (M+1); IR (KBr): 1637 (CO) cm⁻¹; ¹H NMR (200 MHz, CDCl₃): δ 2.57 (s, 3H, CH₃), 2.68 (s, 3H, CH₃), 3.85 (s, 3H, OCH₃), 3.92 (s, 3H, OCH₃), 4.73-4.86 (m, 2H, CH₂), 4.93-4.97 (m, 1H, CH), 6.34 (s, 1H, CH), 6.48 (d, 1H, J = 8.4 Hz, ArH), 6.90 (d, 1H, J = 8.6 Hz, ArH), 7.29 (d, 1H, J = 8.4 Hz, ArH), 7.48 (d, 1H, J = 8.6 Hz, ArH); HRMS calcd. for C₂₄H₂₀N₂ 336.1627, Found 336.1599.

Biological assay

PTP-1B enzyme inhibitor Assay

The effect of test compounds on protein tyrosine phosphatase was studied by pre-incubating 100 μM of the test chemicals in the reaction system for 10 minutes and the residual protein tyrosine phosphatase activity determined according to the method of Goldstein et al. Activity of PTPase was evaluated using p-nitrophenylphosphate (PNPP) as substrate. Assay mixture was made up to 1 mL containing 10mM PNPP in 50 mM HEPES buffer (pH 7), with 1 mM EDTA and DTT. The reaction was stopped by the addition of 500 μL of 0.1 N NaOH and absorbance was determined at 410 nm. A molar extinction coefficient of 1.78 x 10⁴ M⁻¹ cm⁻¹ was used to calculate the concentration of p-nitrophenolate ions produced in the reaction mixture.

Antipromastigote activity.

Luciferase transfected L. donovani promastigotes (MHOM/IN/80/Dd-8, obtained from Imperial College, London), which are more stable under the influence of G 418, were maintained at 25 ± 1 °C in medium 199 (Sigma Chemical, USA) supplemented with 10% foetal calf serum (Gibco). The in vitro effect of compounds on the growth of promastigotes was assessed by monitoring the luciferase activity of viable cells after treatment. The transgenic promastigotes of late log phase were seeded at 5 x 10⁵/100 μl medium 199/well in 96-well flat-bottomed microtitre (MT) plates (CELLSTAR) and incubated for 72 h in medium alone or in the presence of serial dilutions of drugs (0.25–10 μg/ml) in DMSO. Parallel dilutions of DMSO were used as controls. After incubation, an aliquot (50 μl) of promastigote suspension was aspirated from each well of a 96-well plate and mixed with an equal volume of Steady Glo(R) reagent (Promega) and luminescence was measured by a luminometer. The values were expressed as relative luminescence unit (RLU).
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Percentage inhibition = \(N-n/N \times 100\); where \(N\) is average relative luminescence unit (RLU) of control wells; \(n\) is average RLU of treated wells.

2.7 References


