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The first synthesis of podocarflavone A and its analogs and evaluation of their antimycobacterial potential against *Mycobacterium tuberculosis* with the support of virtual screening

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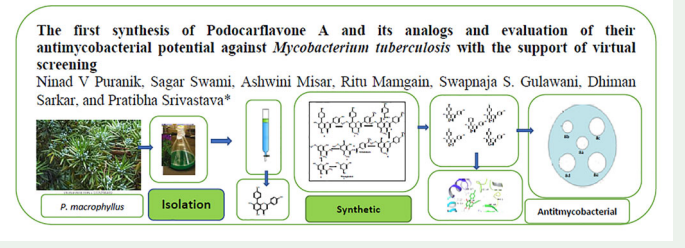
ABSTRACT

The first synthetic route developed for Podocarflavone A reported from *Podocarpus macrophyllus* and its analogs in 7 steps. Computational analysis for binding with the pantothenate kinase (3AVO) of *Mycobacterium tuberculosis* showed their docking score (ds) in the range of −8.9 to −9.3 Kcal/mol. MD simulations delineated the stability of the protein-ligand complexes in the TIP3P model. MMGBSA and MMPBSA values of **8d** were −42.46 Kcal/mol and −14.58 Kcal/mol, respectively. Further in-vitro antitubercular screening of compounds **8a**, **8d**, and **8e** against *M. tuberculosis* H37Ra using XRMA protocol exhibited promising antimycobacterial activity with IC_{50} values 21.82 μg/mL, 15.55 μg/mL, and 16.56 μg/mL, respectively. Compounds **8a**, **8d**, and **8e** showed antibacterial activity with IC_{50} values 41.56 μg/mL, 24.72 μg/mL, and 72.45 μg/mL respectively against the *Staphylococcus aureus*. **8a** and **8d** showed inhibition with IC_{50} values 39.6 μg/mL and 27.64 μg/mL, respectively, against *Bacillus subtilis*. The present study could help in the further development of lead molecules against tuberculosis.



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1. Introduction

Natural products are a dynamic source for innumerable novel pharmaceutical agents (Ahmad et al. 2016, 2017; Newman and Cragg 2016). Their isolation from natural resources is essential for their identification, characterization, and evaluation of biological potential. Every time, their isolation from the plants causes overexploitation of medicinal plants. With natural product synthesis, potential natural products can be available throughout the year (Maier 2015). The flavonoid class of compounds is prevalent among natural products (Crozier et al. 2009).

Podocarflavone A is an 8-aryl flavone reported from the leaves and twigs of plant *Podocarpus macrophyllus maki* and tested for different activities (Qiao et al. 2014). This plant belongs to the Podocarpaceae family and is distributed over tropical and subtropical regions of eastern Asia and Australia. The structures of some of the compounds isolated from this plant are presented in the following figure (Figure 1). Thus, considering the promising biological activities, we contemplated developing a synthetic protocol of podocarflavone A.

Tuberculosis (TB) is an acute infectious disease worldwide, and the causative agent is *Mycobacterium tuberculosis* (Mtb) (WHO 2020a). In 2019, 10 million people got infected, and 1.4 million people died from TB, though it is curable and preventable. An estimated 58 million people have been saved from TB. A complete cure of TB is still beyond reach, and the emergence of new pandemics such as COVID-19 can create hurdles to accomplish it. The DOTS (Directly Observed Treatment, Short Course) therapy announced by the World Health Organization (WHO) has shown an immense effect in achieving more than 90% treatment rates. However, the lengthy period (6–9 months) of the DOTS therapy and reoccurring gene mutations in pathogenic strains became the prime cause of drug resistance (WHO 2020b). Hence, the burst-out cases of multi-drug resistance (MDR) and extensive drug resistance (XDR) have

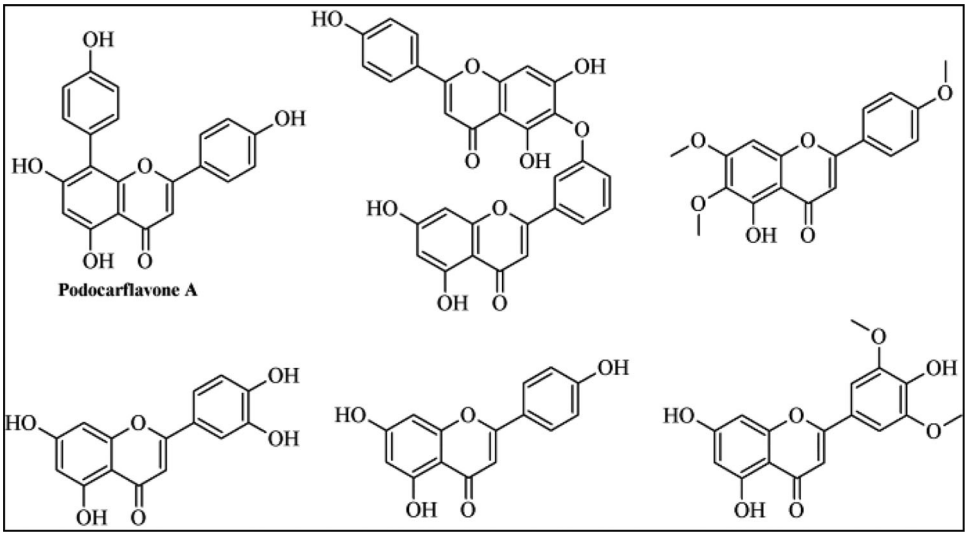


Figure 1. Structures of compounds isolated from *Podocarpus macrophyllus*.

emerged in recent years. The continuous search for potential new molecules with reduced toxicity can eradicate TB from the world.

In the present study, we have reported the total synthesis of natural product podocarflavone A and its analogs and evaluated them for in-vitro antitubercular screening Singh et al. 2011. The essential targets of Mtb are enzymes of the cell wall. Therefore, docking studies of these molecules' with cell wall enzyme pantothenate kinase (**PDB 3AVO**) carried out to understand their interactions (Chetnani et al. 2011). MMGBSA, MMPBSA approach determined the thermodynamic stability of the receptor-ligand complex, and MD simulations studies of 50 ns demonstrated the stability of the complex in the TIP3P model.

2. Results and discussion

Retrosynthetic analysis of podocarflavone A was planned, which confirmed 8-bromo flavone (**6)** is a critical scaffold to synthesize the target molecule **(8a)**. It could be possible either by bromination of suitably substituted flavone **(5) (Route 1**) or by the cyclization of suitably substituted bromo chalcone **(5)** as shown in **Route 2**.

2.1. Retrosynthetic analysis

Retrosynthesis **Route 1** involves the demethylation (Figure 2) of its methoxy analog (**7a**), which can be synthesized from the Suzuki cross-coupling reaction (Hooshmand et al. 2019) of 4-hydroxy phenylboronic acid with 8-bromo-5, 7-dimethoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one **(6)**. The 8-bromo flavone (**6**) intermediate can be prepared from bromination of 5,7-dimethoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one (**5**), which further can be obtained from cyclization of substituted chalcone i.e., 1-(4,6-dimethoxy-2-hydroxyphenyl)-3-(4-methoxyphenyl)prop2-en-1-one (**4**) (Arai et al. 2017). The chalcone can be synthesized using the aldol condensation of 1-(4,6-dimethoxy phenyl-2-hydroxy)- ethanone (**3**) with anisaldehyde using sodium hydroxide as a base (Gurung et al. 2009). Intermediate 4, 6-dimethoxy-2-

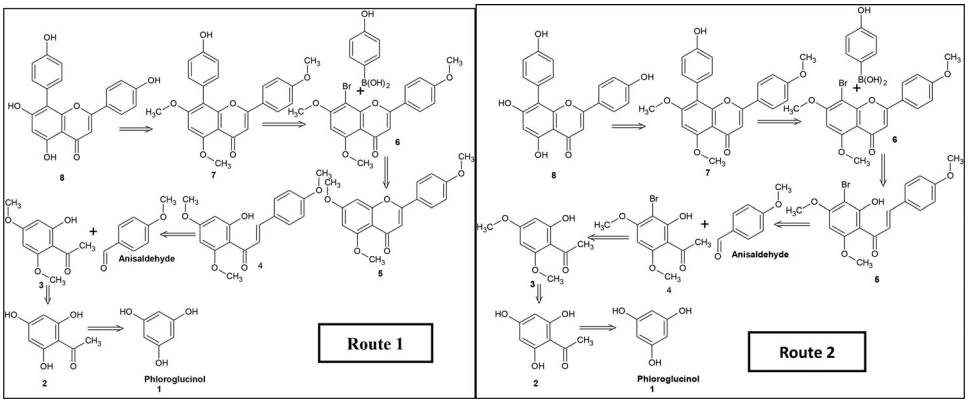
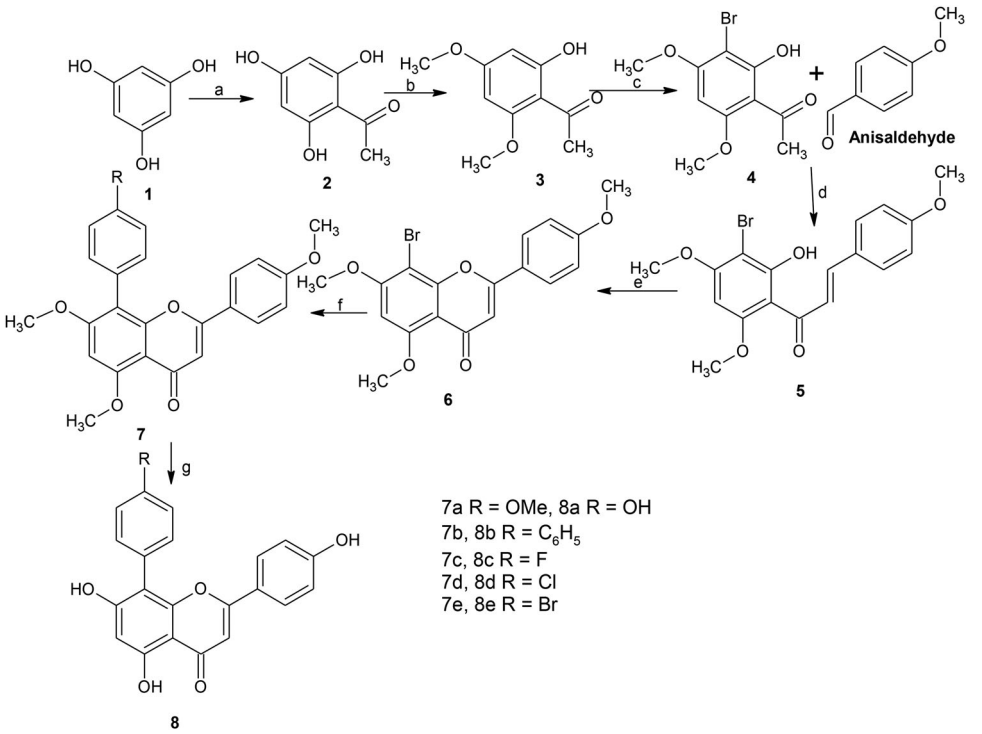


Figure 2. Retrosynthetic route (Route1 and Route 2) considered for the synthesis of natural product Podocarflavone A.



Scheme 1. Synthesis of podocarflavone A and its analogues; Reagents: (a) $AlCl_3$, acetyl chloride, nitrobenzene: DCM (1:1), 0°C-90°C, 2 h; (b) DMS, K_2CO_3, acetone, reflux, 8 h; (c) Br_2, acetic acid, 0°C to RT, 1 h; (d) NaOH, ethanol, RT, 72 h; (e) I_2, DMSO,100°C, 1 h; (f) substituted phenylboronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, DMF, 110°C, 6-12 h; (g) BBr_3, DCM, RT, 12 h.

hydroxyacetophenone (**3**) can be prepared (Sheng et al. 2017) from acylated phloroglucinol **(2)**, which can be synthesized by acylation of phloroglucinol using the reported method (Zhou et al. 2017).

Route 1 could not succeed due to the formation of 6, 8-dibromo flavone instead of monosubstituted 8-bromo flavone even on applying various parameters. This result incited us to consider retrosynthetic route **2** for the preparation of the targeted natural molecule.

Scheme 1 represents a detailed protocol for the synthesis of podocarflavone A and its analogs via **Route 2**. In this route, bromination, chalcone preparation, cyclization, and Suzuki coupling are the key steps. The acylation of phloroglucinol using $AlCl_3$, acetyl chloride in dichloromethane, and nitrobenzene delivered acetylated phloroglucinol (**2**). The yield was 75% (Zhou et al. 2017). Selective O-methylation (Sheng et al. 2017) of **2** using dimethyl sulfate, K_2CO_3, in acetone produced 4,6-dimethoxy-2-hydroxyacetophenone (**3**) with a 90% yield. Bromination of intermediate **3** in the presence of Br_2/acetic acid at 0 °C elicited into 3-bromo-4,6-dimethoxy-2-hydroxyacetophenone (**4**) with an 80% yield (Cechinel-Filho et al. 1996). This step provided **4** having bromo substituent at the desired position. Synthesis of 1-(3-bromo-4, 6-dimethoxy-2-hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one **(5)** was done by base-catalyzed aldol condensation (Bandgar et al. 2012) of **4** with anisaldehyde, and the chalcone (**5**)

obtained with 95% yield. The cyclization of chalcone (**5**) in the presence of iodine and dimethylsulfoxide on heating resulted into 8-bromo-5,7-dimethoxy-2-(4-methoxyphenyl)-4H-benzopyran-4-one (**6**) (Gurung et al. 2009). The Suzuki cross-coupling reaction (Pan et al. 2017; Hooshmand et al. 2019) was performed for the C-C bond formation at the 8th position of the intermediate **6** with 4-hydroxyphenyl boronic acid produced methylated natural product Podocarflavone A (**7a**). The desired natural molecule Podocarflavone A obtained by deprotection of methyl group using BBr_3/DCM/12h (Ravishankar et al. 2016). Different 4-substituted phenylboronic acids were used to prepare the analogs of Podocarflavone A in the Suzuki coupling step (**Scheme-1**). Podocarflavone A and analogs were characterized by spectroscopic analysis. The spectral data were cross-checked with the natural product reported for confirmation, and it was found in agreement with the isolated natural product. Podocarflavone A (**8a**) was obtained after synthesis as the white powdered solid. The LC-MS of **8a** displayed a single peak and confirmed the mass of m/z 363.0. The FTIR spectra also showed two significant peaks at 3310 cm^{-1} and 1651 cm^{-1} for hydroxyl and carbonyl groups respectively. The ^{1}H NMR (400 MHz, DMSO-d_6) spectrum displayed a singlet δ 6.37 (s, 1H, 6-H), and another singlet for proton 3-H of ring A merged with a doublet of 3′,5′ H of the 4-hydroxyphenyl ring (**C**) and came as multiplet 6.78-6.80 (m, 3H, 3-H, 3′,5′ H). The three dublets 6.84 (d, J = 8.4 Hz, 3″, 5″ H), 7.25 (d, J = 8.8 Hz, 2″, 6″ H), 7.62 (d, J = 8.8 Hz, 2′, 6′ H), belong to aromatic protons of ring C and ring D respectively. Elegantly, we also got separate peaks for four hydroxyl groups, which are present at 7th position of B ring at 9.49 s, OH, 7-OH), 4th position of ring D 10.33 (s, OH, 4″) , 4th position of ring C at 10.66 (s, OH, 4′), and 5th position of ring B at 13.11 (s, OH, 5-OH) respectively. Table S1 showed the comparative analysis of ^{1}H NMR, ^{13}CNMR, and IR spectra. The ^{13}C NMR displayed signals for 21 carbons, which contains the signals of a carbonyl carbon (δ 182.7) and 20 sp2 carbons. The spectral analysis confirmed the total synthesis of Podocarflavone A and was found in agreement with the isolated Podocarflaone A (Qiao et al. 2014).

2.2. Antitubercular activity

Amongst the tested compounds, compounds **8a**, **8 b**, **8c**, **8d,** and **8e** exhibited promising antimycobacterial activity with IC_{50} values 21.82 μg/mL, 4.84ug/ml with 24.84ug/ml , 22.54ug/ml, 15.55 μg/mL, and 16.56 μg/mL, respectively (Table S2). The compounds **8d** and **8e** were found more potent than the natural Podocarflavone A to inhibit Mtb. The results showed that the conversion of the hydroxyl group with the methoxy group restrict their inhibitory property. Replacement of the hydroxyl group at 4th position in ring D of **8a** with chloro or bromo group enhances its inhibitory potential against Mtb. We observed the same kind of results when we checked with dihydrorugosaflavonoids derivatives (Puranik et al. 2018).

2.3. Antibacterial activity

During the screening, three compounds **8a**, **8d**, and **8e**, showed potential against Gram + ve bacteria while there was no inhibitory activity towards Gram-ve bacteria.

Compound **8a** showed IC_{50} at 41.56 μg/mL 39.6 μg/mL against *S. aureus* and *B. subtillis,* respectively. **8d** showed IC_{50} at 24.72 μg/mL and 27.64 μg/mL against *S. aureus* and *B. subtillis,* respectively. **8e** showed IC_{50} at 72.45 μg/mL against *S. aureus*. Data are presented in Table S3.

2.4. Molecular modelling

Pantothenate kinase (3AVO) is an essential enzyme for Mtb and catalyzes the ATP-based phosphorylation of pantothenate. It is a crucial step in the biosynthetic pathway of coenzyme A from pantothenic acid. All the compounds were docked at the binding pocket to identify ligand-receptor interactions. Podocaflavone A and its analogs displayed strong binding properties to PanK with docking scores −7.2 to −9.3 Kcal/mole. The docking image of **8a, 8c, 8d,** and **8e** are displayed in Figure S1. The interacting residues and docking scores are presented in Table S2. Mostly all compounds showed hydrogen-bonded interactions with Arg 238. Essential residues are Asp 129, Lys147, Tyr 235, Arg 238, Asn 277 of the active binding pocket. All the compounds are surrounded by these amino acid residues and displayed interactions (Figure S1). Based on their binding profiles, these compounds are screened for anti-TB activity. Only compounds **8a**, **8c**, **8d**, and **8e** showed inhibitory activity. Therefore, the MD simulations of active compounds (**8a**, **8c**, **8d**, and **8e**) were performed.

2.5. Molecular dynamics simulations

The docked complexes of the compounds **8a**, **8c**, **8d**, and **8e** were subjected to analyze their stability in the TIP3P water model for 50 ns. The MD simulations on minimized and equilibrated complexes of **8a**, **8c**, **8d**, and **8e** were subjected to MD simulations for 50 ns Wang et al. 2006. RMSD versus time graph of the trajectories displayed in Figure S2. The complexes' trajectories showed stability in the MD simulations, and RMSD values are determined (Table S4).

2.6. MMGBSA and MMPBSA analysis

The thermodynamic stability of proteins was determined by MMGBSA and MMPBSA analysis using Amber 20 Software Miller et al. 2012. The free energy values were calculated with the help of the mmpbsa.py script of Amber 20 by using a number of frames generated during MD simulations. It gave information about the thermodynamic stability of the complex. The MMPBSA and MMGBSA values of complexes are presented in Table S4.

3. Experimental section

All the procedures for the intermediates from 2-8 are prepared and characterized by NMR. Their methodology is given in supplementary file 1 **(Supplementary file 1).** It

also contains Figure S1 and S2, Tables S1–S4, the Mass (LC-MS), ^{1}H NMR, and ^{13}C NMR spectra of **7a-7e** and **8a-8e.**

4. Conclusion

The flavonoids are widely distributed in fruits and vegetables and are essential for human health. Therefore, we have reported the first synthetic route involving bromination followed by chalcone preparation, cyclization, and Suzuki coupling as the key steps to synthesize natural product Podocarflavone A, which is reported from *Podocarpus macrophyllus*. The natural product **8a** was successfully prepared in 7 steps. During the synthesis, their analogs are prepared following the same route. The halogenated Podocarflavone A showed better results in comparison to the natural product **8a**. The computational studies and in-vitro antimycobacterial screening results confirm inhibitory activity against Mtb. These molecules can serve to discover the lead molecules as antitubercular agents.

Disclosure statement

No potential conflict of interest was reported by the authors.

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