Research Article

Synthesis of ($\pm$)-Pisonivanone and Other Analogs as Potent Antituberculosis Agents

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A new class of alkylated chalcones and flavanones was synthesised and screened for antituberculosis, antioxidant, and cytotoxic activities. The desired compounds were synthesised using methyl substituted 2-hydroxyacetophenone as a key intermediate. The acetophenone derivative having methyl substitution was prepared in turn from methylated phloroglucinol by formylation (by Vilsmeier-Haack reaction), followed by reduction with Wolf-Kishner approach, and finally acetylation was involved. Among 17 compounds, compound **5** and compound **4a** inhibited *M. tuberculosis* at minimum inhibitory concentration (MIC) in the range between 25 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$. The remaining other 15 compounds also potently inhibited *M. tuberculosis* at MIC in range between 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$. Some of these compounds also showed moderate antioxidant and cytotoxic activities.

1. Introduction

A survey of the literature revealed that many flavonoids benefit human health [1]. Among these large class of compounds some of the alkylated flavonoids isolated from plants also act as antimalarial [2], antioxidant [3], anticancer [4–6], anti-inflammatory [7], and antimicrobial agents [8] like unsubstituted flavonoids. Recently antitubercular chromones and flavonoids [9] having methyl substitution were isolated from *Pisonia aculeate*, which belongs to Nyctaginaceae family. Among these the titled compound pisonivanone was reported as a potent antituberculosis compound (MIC = 12.5 $\mu\text{g/mL}$). So in order to develop the activity profile of the methyl substituted flavonoids, an attempt was made to synthesize pisonivanone for the first time and other new chalcones and flavanones. These synthesized compounds were screened for some biological activities like antioxidant, cytotoxic, and antituberculosis. The scheme for the synthesis of Pisonivanone and other analogs was given below (Scheme 1).

2-Hydroxy-3-methyl-4,6-dimethoxy acetophenone (**1**) was synthesised in different ways [10]. In the present work,

we aimed to synthesise the key intermediate acetophenone (**1**) from phloroglucinol using regular conventional methods like methylation [11], formylation [12], Wolf-Kishner reduction, and acetylation [12] with better yields (Scheme 2). All the intermediates were confirmed by comparing the spectral data and melting points with the literature.

Chalcones are versatile molecules and intermediates for synthesis of different heterocyclic compounds [13]. Chalcones have been synthesised in different ways [14, 15]. The chalcones (**3a–h**) were synthesised by Claisen-Schmidt reaction. In this reaction, condensation was between acetophenone (**1**) and substituted aromatic aldehydes (**2a–h**) in the presence of aqueous KOH and ethanol at room temperature.

Flavanones (Compound **4a–h**) were prepared on cyclisation of chalcones (Compounds **3a–h**, resp.) in the presence of alcoholic H_2SO_4 . Initially, chalcones were dissolved in alcoholic H_2SO_4 and refluxed at 60°C for 24 hrs. After cooling, alcohol was removed under vacuum. When ice cold water added to the reaction, mass pale reddish colour solids were separated. These solids were extracted with ethyl acetate. This ethyl acetate layer dried over sodium sulphate. This ethyl

SCHEME 1: Reagents and conditions: (i) KOH/EtOH, 24 h. (ii) EtOH, H_2SO_4 , 60°C , 24 h.

SCHEME 2: Reagents and conditions: (i) DMS/Acetone, K_2CO_3 , 70°C /6 h, (ii) DMF/ POCl_3 , 0°C /1 h, (iii) $\text{NH}_2\text{NH}_2\cdot\text{H}_2\text{O}/\text{KOH}$, ethylene glycol, $80-90^\circ\text{C}$ for 2 h followed by heating at 130 to 140°C for 2 h, (iv) $\text{CH}_3\text{COCl}/\text{AlCl}_3$, MDC, 0°C /2 h.

acetate layer was concentrated and washed with diethyl ether to remove adherent chalcone.

Finally, the targeted compound **5** (pisonivanone) was synthesised from Compound **4a** on demethylation. Pyridinium hydrochloride was added to Compound **4a**, at inert conditions in 1:10 ratio and refluxed for 1 hour at 200°C . Then reaction mixture poured in ice cold water and extracted with ethyl acetate. This ethyl acetate layer dried over sodium sulphate and on column chromatography with ethyl acetate, and hexane pure pisonivanone was separated (yellow crystalline solid, yield 55%) (see Scheme 3).

2. Discussions

Based on the literature, all these synthesised Compounds **3a-h**, **4a-h**, Compound-5 (pisonivanone) were screened for their biological activities like antitubercular, antioxidant, and cytotoxicity. All these methyl substituted flavonoids were showed potent antitubercular activity against *M. tuberculosis* strain (H37Rv). Possibly the C-8 methyl group in basic skeleton of the molecules plays an important role in the antimycobacterial activity. Among 17 compounds Compound **5** showed minimum inhibitory concentration (MIC)

at $25\text{ }\mu\text{g/mL}$ and Compound **4a** inhibited *M. tuberculosis* at minimum inhibitory concentration (MIC) $\geq 50\text{ }\mu\text{g/mL}$. The remaining other 15 compounds also potently inhibited *M. tuberculosis* at MIC in range between $50\text{ }\mu\text{g/mL}$ and $100\text{ }\mu\text{g/mL}$. These results suggest that the methyl substituted flavonoids may consider as potent antituberculosis agents, and there is a scope to improve the activity further by preparing different possible analogs. The results given in Tables 2 and 3 for chalcones and flavanones showed the compounds have moderate antioxidant and cytotoxic activities.

3. Experimental

3.1. Materials and Methods. Phloroglucinol and other chemicals used were of AR grade (Merck). Melting points were taken in open glass capillary by using Remi melting point apparatus and were uncorrected. ^1H NMR spectra were recorded on Bruker 400 MHz and ^{13}C NMR was on Bruker 100 MHz spectrophotometers. Mass spectra recorded on LC-MS Agilent LC-1100 series.

3.1.1. Synthesis of 2,4,6-Trimethoxy Phloroglucinol. A solution of anhydrous phloroglucinol (50 g, 0.396 mole) in

SCHEME 3

dry acetone (700 mL) is refluxed for 6 h at 60°C under anhydrous conditions with dimethyl sulphate (112.10 mL, 148 g, 1.17 mole) in presence of potassium carbonate (280 g, 2.02 mole). The acetone layer was filtered and distilled. The residue on column chromatography with hexane and ethyl acetate (95:5) trimethoxy phloroglucinol separated. (% of yield 94, white solid, M.P—52–54°C.)

3.1.2. Synthesis of 2,4,6-Trimethoxy Benzaldehyde. Dimethyl formamide (36.8 mL, 1.3 eq, 0.4797 mole) is added to trimethoxy phloroglucinol (62 g, 0.369 mole, 1 eq) and cooled (0°C.) Phosphorous oxychloride (153.33 mL, 65.63 gm, 1.16 eq) is added drop wise to this cooling mixture slowly under anhydrous conditions. The mixture is allowed to stand for 1 h at room temperature. The reaction mixture poured into crushed ice and basified with 8 N KOH. The filtered solid dissolved in ethyl acetate and treated with brine. The ethyl acetate layer dried over sodium sulphate and concentrated. (% of yield—91.6, yellow viscous solid, M.P—119–120°C.)

3.1.3. Synthesis of 3-Methyl-2,4,6 Trimethoxy Phloroglucinol. 2,4,6-Trimethoxy benzaldehyde is dissolved in ethylene glycol and hydrazine hydrate is added. Powdered KOH portion wise added to the reaction mixture and heated to 80–90°C for 2 h then foaming is formed. The reaction mixture heated to 135–145°C for another 2 h. Then the reaction mixture is poured into ice cold water, acidified with dil HCl, and extracted with hexane. Hexane layer is washed with sodium carbonate, water, and brine. This hexane layer dried over sodium sulphate and concentrated. (% of yield 80, colour less liquid.)

3.1.4. Synthesis of 2-Hydroxy-3-methyl-4,6-dimethoxy Acetophenone (1). A solution of 3-methyl-2,4,6 Trimethoxy phloroglucinol (55 g, 0.302 mole) in absolute ether (450 mL) is added to a cold solution of anhydrous aluminum chloride (86.39 g, 0.64 mole, 2.2 eq) in absolute ether (100 mL). The cooled (0–5°C) and stirred solution is treated with acetyl chloride (25.21 mL, 0.319 mole, 1.2 eq), added dropwise during 1 hr. The mixture is stirred for 4 hr more. The whole reaction is done under anhydrous condition. The mixture is left overnight at room temperature and treated in cold with crushed ice. It is acidified with 5 N HCl and extracted with ethyl acetate. This ethyl acetate layer dried over sodium

sulphate and concentrated. (% of yield 85, light green color crystals, M.P—90–92°C.)

3.1.5. General Method for Synthesis of Chalcones (Compound 3a–h). To a solution of acetophenone (0.5 g, 0.00238 mole) and substituted benzaldehydes (2a–h) (1 eq) in ethanol (10 mL) is added aqueous potassium hydroxide (0.599 g, 4.5 eq in 0.5 mL water). The mixture is stirred at room temperature for 24 h. To this ice cold water added and separated solid was filtered. The separated solid was dissolved in ethyl acetate, dried over sodium sulphate, and concentrated under vacuum.

3.1.6. Spectral Data of Synthesized Compounds. Compound 3a. It is having the molecular formula $\text{C}_{19}\text{H}_{20}\text{O}_5$ which showed $(\text{M} + \text{H})^+$ at 329 in LC-MS. So the molar mass of the Compound 3a is 328 g/mol. Yield—85%, M.P—157–160°C.

^1H NMR (CDCl_3 , 400 MHz, δ in ppm): 3H, s, 3.991 (Ar-OCH₃), 3H, s, 3.932 (Ar-OCH₃), 3H, s, 3.917 (Ar-OCH₃), 1H, s, 14.045 (Ar-OH), 2H, d, 7.98 (Ar¹-H), 1H, dd, 7.71 (Ar¹-H), 1H, m, 7.4 (Ar¹-H), 1H, d, 7.05 (Ar¹-H), 1H, d, 7.1 (C=C-H), 1H, d, 6.3 (C=C-H).

^{13}C NMR (CDCl_3 , 100 MHz, δ in ppm): 192.72 (C=O), 7.32 (ArCH₃), 56.1 (Ar-OCH₃), 55.8 (Ar-OCH₃), 55.69 (Ar¹-OCH₃), 163.6, 162.9, 161.5 (Ar-C-O), 158.32 (Ar¹-C), 87.65 (Ar-C), 105.6, 104.2 (Ar-C), 120.87, 111.88 (C=C), 137.28, 131.93, 128.96, 127.77, 123.30 (Ar¹-C).

Compound 3b. It is having the molecular formula $\text{C}_{19}\text{H}_{20}\text{O}_5$ which showed $(\text{M} + \text{H})^+$ at 329 in LC-MS. So the molar mass of the compound 3b is 328 g/mol. Yield—80%, M.P—125–130°C.

^1H NMR (CDCl_3 , 400 MHz, δ in ppm): 1H, d, 14.03 (Ar-OH), 1H, d, 7.8 (Ar¹-H), 1H, d, 7.7 (Ar¹-H), 1H, s, 5.99 (Ar-H), 1H, s, 7.29 (Ar¹-H), 1H, m, 7.33 (Ar¹-H), 1H, d, 7.21 (C=C-H), 1H, d, 6.9 (C=C-H), 3H, s, 3.9 (Ar¹OCH₃), 3H, s, 3.9 (Ar-OCH₃), 3H, s, 3.8 (Ar-OCH₃), 3H, s, 2.03 (Ar-CH₃).

^{13}C NMR (CDCl_3 , 100 MHz, δ in ppm): 193.00 (O=C), 164.27, 163.65 (Ar-C-OCH₃), 161.13 (Ar-OH), 159.93 (Ar¹-OCH₃), 141.72 (C=C-Ar), 128.35 (C=C-C=O), 86.43 (Ar-C), 106.40 (Ar-C-CH₃), 106.22 (Ar-C-C=O), 137.1, 129.80, 120.87, 115.51, 113.63 (Ar¹-C), 55.84, 55.49 (Ar-OCH₃), 55.28 (Ar¹-OCH₃), 7.23 (Ar-CH₃).

Compound 3c. Compound having the molecular formula $C_{21}H_{24}O_7$ is showed molecular ion peak at 389.2 (Neg) in LC-MS. Molecular weight of the compound **3c** is 388 g/mol. Yield—86%, M.P—200–205°C.

1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 1H, s, 14.09 (Ar-OH), 3H, s 3.93 (Ar¹-OCH₃), 6H, s 3.9 (Ar¹-OCH₃), 3H, s 3.9 (Ar-OCH₃), 3H, s 3.8 (Ar-OCH₃), 3H 2.03 (Ar-CH₃), 1H, s 5.99 (Ar-H), 2H, s 6.83 (Ar¹-H), 1H d 7.7 (C=C-H), 1H, d 7.69 (C=C-H).

^{13}C NMR ($CDCl_3$, 100 Hz, δ in ppm): 192.75 (O=C), 164.29, 163.57, (Ar-C-OCH₃), 161.02 (Ar-OH), 153.44 (Ar¹-OCH₃), 141.97 (Ar¹-OCH₃), 141.97 (Ar¹-OCH₃), 140.21 (C=C-Ar), 127.35 (C=C-C=O), 86.43 (Ar-C), 106.40 (Ar-C-CH₃), 106.24 (Ar-C-C=O), 131.24, (Ar¹-C), 105.72 (Ar¹-C), 105.72 (Ar¹-C), 60.95, 56.18 (Ar-OCH₃), 55.79, 55.49 55.28 (Ar¹-OCH₃), 7.23 (Ar-CH₃).

Compound 3d. It is having the molecular formula $C_{18}H_{17}O_4Cl$ which showed molecular ion ($M - H$)⁻ peak in negative mode at 331 in LC-MS. So the molar mass of the compound **3d** is 332 g/mol. Yield—70%, M.P—185–190°C.

1H NMR (400MHz, δ in ppm, $CDCl_3$): 1H, s,14.00 (Ar-OH), 3H, s, 3.95 (Ar-OCH₃), 3H,s, 3.980 (Ar-OCH₃), 3H,s, 2.037 (Ar-CH₃), 1H,s, 5.92 (Ar-H), 2H,d 7.37 (Ar¹-H), 2H,d, 7.53 (Ar¹-H), 1H d, 7.7 (C=C-H), 1H,d,7.8 (C=C-H).

^{13}C NMR (100MHz, δ in ppm, $CDCl_3$): 192.73 (C=O), 164.28, 163.76 (Ar-C-OH₃), 161.11 (Ar-C-OH), 86.40 (Ar-C), 106.32 (Ar-C-CH₃), 106.24 (Ar-C-C=O), 140.33 (-C=C-Ar), 128.50 (-C=CH), 135.74 (Ar¹-Cl), 134.22 (Ar¹-C),129.39 (Ar¹-C-H), 128.50 (Ar-C-H), 137.45(CH=C), 127.85 (C=C-H).55.85, 55.52 (Ar-O-CH₃), 7.24 (Ar-CH₃).

Compound 3e. It is having the molecular formula $C_{18}H_{17}O_4F$ which showed molecular ion peak in positive mode ($M + H$)⁺ at 317 in LC-MS. So the molar mass of the compound **3e** is 316 g/mol. Yield—75%, M.P—165–170°C.

1H NMR (400 MHz, δ in ppm, $CDCl_3$): 1H,s, 14.01 (Ar-OH), 1H,s, 5.985 (Ar-H), 3H,s, 3.98 (Ar-O-CH₃), 3H,s, 3.89 (Ar-O-CH₃), 3H,s, 2.03 (Ar-CH₃), 1H,d, 8.00 (C=C-H), 1H,d, 7.84 (C=C-H), 1H,m, 7.5 (Ar¹-H), 1H,m, 7.3 (Ar¹-H), 1H,m, 7.14 (Ar¹-H), 1H, m, 7.09 (Ar¹-H).

^{13}C NMR (100 MHz, δ in ppm, $CDCl_3$): 193.04(C=O), 106.42 (Ar-C-CH₃),164.34 (Ar-O-CH₃), 86.44 (Ar-C-H), 163.80 (Ar-O-CH₃),162.97 (Ar-C-OH),106.21 (Ar-C-C=O), 55.77,55.53 (Ar-OCH₃), 7.26 (Ar-CH₃),161.25,160.45 (Ar¹-C-F),123.78,123.90 (Ar¹-C),131.18,131.09 (Ar¹-C), 130.79, 130.72 (Ar¹-C), 129.76, 129.73 (Ar¹-C), 116.33, 116.11 (Ar¹-C), 134.46(HC=C), 124.38(HC=C).

Compound 3f. It is having the molecular formula $C_{18}H_{17}O_4Br$ which showed molecular ion peak in positive mode ($M + H$)⁺ at 378 in LC-MS. So the molar mass of the compound **3f** is 377 g/mol. Yield—60%, M.P—175–180°C.

1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 1H, s 13.99 (Ar-OH), 3H, s 2.032 (Ar-CH₃), 3H, s 3.93 (Ar-OCH₃), 3H, s 3.89 (Ar-OCH₃), 1H, d, 7.68 (Ar-H), 2H, d, 7.5 (Ar¹-H), 2H, d, 7.44 (Ar¹-H), 1H, d, at 7.85 (O=C-CH=C), 1H, d, 5.98 (C=C-H).

TABLE 1: Media preparation for antituberculosis activity.

MI of stock (mg/L)	L J Fluid (mL)	Final concentration(mg/L)
2 mL of 2000	20 mL	200 mg/L
1 mL of 2000	20 mL	100 mg/L
0.5 mL of 2000	20 mL	50 mg/L
0.25 mL of 2000	20 mL	25 mg/L
0.125 mL of 2000	20 mL	12.5 mg/L

^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm): 192.70 (Ar-C=O), 7.23 (Ar-CH₃), 106.26 (Ar-C-CH₃), 164.31 (Ar-C-OCH₃), 86.42 (Ar-C-H), 163.78 (Ar-C-OCH₃), 106.33 (Ar-C-C=O), 161.12 (Ar-C-OH), 55.84 (Ar-OCH₃), 55.50 (Ar-O-CH₃), 134.68 (Ar¹-C<), δ at 129.60 (Ar¹-C-H), 128.65 (Ar¹-C-H), 132.08 (Ar¹-C-Br), 129.60 (Ar¹-C-H), 124.04 (Ar¹-C-H), 140.32 (O=C-CH=C), 128.65 (C=C-H).

Compound 3g. It is having the molecular formula $C_{20}H_{22}O_6$ which showed molecular ion peak in negative mode ($M - H$)⁻ at 357.2 so the molar mass of the compound **3g** is 358.2 g/mol. Yield—75%, M.P—130–135°C.

1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 1H, s 14.18 (Ar-OH), 3H, s 2.03 (Ar-CH₃), 6H, s 3.9 (Ar-OCH₃), 1H, s, 5.98 (Ar-H), 1H, d, 7.21 (O=C-CH=C), 1H, d, 6.89 (C=C-H), 2H, m, 7.7 (Ar¹-H), 1H, s 7.11 (Ar¹-H), 3H, s 3.92 (Ar¹-OCH₃), 3H, s, 3.89 (Ar¹-OCH₃).

^{13}C NMR (100MHz, δ in ppm, $CDCl_3$): 192.85 (Ar-C=O), 106.39 (Ar-C-CH₃), 164.27 (Ar-C-OCH₃), 163.43 (Ar-C-OCH₃), 160.99 (Ar-C-OH), 86.46 (Ar-C-H), 106.20 (Ar-C-C=O), 55.98 (Ar-OCH₃)55.89 (Ar-O-CH₃), 125.92 (Ar¹-C<), 110.65 (Ar¹-C-H), 111.31 (Ar¹-C-H), 122.52 (Ar¹-C-H), 151.07 (Ar¹-C-OCH₃), 149.22 (Ar¹-C-OCH₃), 55.79 (Ar¹-OCH₃), 55.47 (Ar¹-OCH₃), 142.18(CH=C), 128.52 (C=C-H).7.25 (Ar-CH₃).

Compound 3h. It is having the molecular formula $C_{18}H_{16}O_4F_2$ which showed molecular ion peak in negative mode ($M - H$)⁻ at 333.2 in LC-MS. So the molar mass of the compound **3h** is 334.2 g/mol. Yield—60%, M.P—135–140°C.

1H NMR ($CDCl_3$, 400 MHz, δ in ppm): 3H, s, 2.03 (Ar-CH₃), 3H, s 3.95 (Ar-OCH₃), 3H, s 3.90 (Ar-H), 1H, s, 7.78 (Ar-OCH₃), 1H, s 13.94 (Ar-OH), 1H, d, 6.89 (O=C-CH=C), 1H, d, 7.21 (C=C-H), 1H, m, 7.7 (Ar¹-H), 1H, m 7.3 (Ar¹-H), 1H, m, 7.1 (Ar¹-H).

^{13}C NMR ($CDCl_3$, 100 MHz, δ in ppm): 7.78 (Ar-CH₃), 106.30 (Ar-C-CH₃), 163.86 (Ar-C-OCH₃), 86.42 (Ar-C-H), 164.31 (Ar-C-OCH₃), 106.27 (Ar-C-C=O), 161.12 (Ar-C-OH), 55.86 (Ar-OCH₃), 55.47 (Ar-O-CH₃), 192.69 (Ar-C=O), 129.0 (Ar¹-C<), 124.99 (Ar¹-C-H), 125.06 (Ar¹-C-H), 133.06 (Ar¹-C-F), 132.98 (Ar¹-C-F), 117.8 (Ar¹-C-H), 139.26 (CH=C), 126.81 (C=C-H).

3.1.7. General Method for Synthesis of Flavanones (Compound 4a–h). To the previously obtained chalcones (**3a–h**) (0.2 g) was refluxed in alcoholic sulphuric acid (10%, 10 mL) for 24 h. The alcohol was distilled off in vacuo and the residue is diluted with water. The separated product was dissolved

in ethyl acetate and dried over sodium sulphate. The ethyl acetate layer was concentrated the residue was washed with ether to remove any adherent chalcone to obtain pure flavanones (**4a-h**).

Compound 4a. It is having the molecular formula $C_{19}H_{20}O_5$ which showed molecular ion peak in positive mode $(M + H)^+$ at 329 in LC-MS. So the molar mass of the compound **4a** is 328 g/mol. Yield—70%, M.P—172–175°C.

1H NMR: ($CDCl_3$, 400 MHz, δ in ppm): 1H, s, 6.27 (Ar-H), 1H, m, 7.19 (Ar^1 -H), 2H, m, 6.7 (Ar^1 -H), 1H, d, 6.8 (Ar^1 -H), 1H, d, 4.82 (O-C-H), 6H, s, 3.86 (Ar-O-CH₃), 3.71 (3H, s, Ar^1 -O-CH₃), 3H, s, 2.16 (Ar-CH₃), 3.06 and 2.88 (1H, d and 1H, d O=C-CH₂).

^{13}C NMR: ($CDCl_3$, 100 MHz, δ in ppm): 7.81 (Ar-CH₃), 106.36 (Ar-C-CH₃), 163.56 (Ar-C-OCH₃), 88.64 (Ar-C-H), 160.12 (Ar-C-OCH₃), 105.98 (Ar-C-C=O), 160.52 (Ar-C-O), 55.92 (Ar-OCH₃), 55.08 (Ar-O-CH₃), 189.83 (Ar-C=O), 129.73 (Ar^1 -C<), 118.01 (Ar^1 -C-H), 159.90 (Ar^1 -C-OCH₃), 111.58 (Ar^1 -C-H), 140.99 (Ar^1 -C-H), 113.63 (Ar^1 -C-H), 55.62 (Ar^1 -OCH₃), 45.75 (O=C-CH₂), 78.28 (>C-H).

Compound 4b. It is having the molecular formula $C_{19}H_{20}O_5$ which showed molecular ion peak in positive mode $(M + H)^+$ at 329 in LC-MS. So the molar mass of the compound **4b** is 328 g/mol. Yield—60%, M.P—166–170°C.

1H NMR: ($CDCl_3$, 400 MHz, δ in ppm): 1H, s, 6.125 (Ar-H), 2H, s, 7.04 (Ar^1 -H), 1H, dd, 6.8 (Ar^1 -H), 1H, dd, 6.98 (Ar-H), 1H, dd, 5.3 (O-C-H), 3H, s, 3.93 (Ar-O-CH₃), 3H, s, 3.93 (Ar-O-CH₃), 3H, s, 3.83 (Ar^1 -O-CH₃), 3H, s, 2.06 (Ar-CH₃), 2.9, and 2.8 (1H, dd and 1H, dd, O=C-CH₂).

^{13}C NMR: ($CDCl_3$, 100 MHz, δ in ppm): 7.81 (Ar-CH₃), 106.3 (Ar-C-CH₃), 161.12 (Ar-C-OCH₃), 88.64 (Ar-C-H), 160.52 (Ar-C-OCH₃), 105.98 (Ar-C-C=O), 163.56 (Ar-C-O), 55.62 (Ar-OCH₃), 56.06 (Ar-O-CH₃), 189.83 (Ar-C=O), 140.99 (Ar^1 -C<), 129.97 (Ar^1 -C-H), 159.90 (Ar^1 -C-OCH₃), 118.01 (Ar^1 -C-H), 111.58 (Ar^1 -C-H), 113.6 (Ar^1 -C-H), 55.27 (Ar^1 -OCH₃), 45.75 (O=C-CH₂), 78.28 (>C-H).

Compound 4c. It is having the molecular formula $C_{21}H_{24}O_7$ which showed molecular ion peak in positive mode $(M + Na)^+$ at 411.2 in LC-MS. So the molar mass of the compound **4c** is 388.2 g/mol. Yield—65%, M.P—164–170°C.

1H NMR: ($CDCl_3$, 400 MHz, δ in ppm): 1H, s, 6.13 (Ar-C-H), 3H, s, 2.06 (Ar-CH₃), 2H, s, 6.69 (Ar^1 -C-H), 1H, dd, 5.3 (Ar^1 -O-C-H), 3H, s, 3.94 (Ar-OCH₃), 3H, s, 3.91 (Ar-OCH₃), 3H, s, 3.8 (Ar^1 -OCH₃), 6H, s, 3.88 (Ar^1 -OCH₃), 1H, dd at δ 2.97 and 1H, dd at δ 2.86 (—O=C-CH₂—).

^{13}C NMR: ($CDCl_3$, 100 MHz, δ in ppm): 7.83 (Ar-CH₃), 105.91 (Ar-C-CH₃), 161.04 (Ar-C-OCH₃), 88.62 (Ar-C-H), 160.54 (Ar-C-OCH₃), 103.03 (Ar-C-C=O), 163.61 (Ar-C-O), 56.20 (Ar-OCH₃), 56.06 (Ar-O-CH₃), 189.85 (Ar-C=O), 138.01 (Ar^1 -C<), 103.03 (Ar^1 -C-H), 153.47 (Ar^1 -C-OCH₃), 153.47 (Ar^1 -C-OCH₃), 134.96 (Ar^1 -C-OCH₃), 103.03 (Ar^1 -C-H), 55.64 (Ar^1 -OCH₃), 55.64 (Ar^1 -OCH₃), 60.83 (Ar^1 -OCH₃), 45.78 (O=C-CH₂), 78.42 (>C-H).

Compound 4d. It is having the molecular formula $C_{18}H_{17}O_4Cl$ which showed molecular ion peak in positive

mode $(M + H)^+$ at 333.2 in LC-MS. So the molar mass of the compound **4d** is 332 g/mol. Yield—60%, M.P—177–180°C.

1H NMR (400MHz, δ in ppm, $CDCl_3$): 3H,s, 2.04 (Ar-CH₃), 3H,s, 3.93 (Ar-OCH₃), 3H,s, 3.90 (Ar-OCH₃), 1H,s, 6.128 (Ar-H), 2H,m, 2.9 (O=C-CH₂), 1H,dd, 5.38 (>C<H), 4H, m, 7.4 (Ar^1 -H).

^{13}C NMR: ($CDCl_3$, 100 MHz, δ in ppm): 7.79 (Ar-CH₃), 106.33 (Ar-C-CH₃), 160.87 (Ar-C-OCH₃), 88.69 (Ar-C-H), 160.58 (Ar-C-OCH₃), 105.33 (Ar-C-C=O), 163.67 (Ar-C-O), 55.63 (Ar-OCH₃), 56.06 (Ar-O-CH₃), 189.47 (Ar-C=O), 134.16 (Ar^1 -C<), 127.21 (Ar^1 -C-H), 128.8 (Ar^1 -C-H), 137.87 (Ar^1 -C-Cl), 128.88 (Ar^1 -C-H), 127.21 (Ar^1 -C-H), 45.56 (O=C-CH₂), 77.74 (>C-H).

Compound 4e. It is having the molecular formula $C_{18}H_{17}O_4F$ which showed molecular ion peak in positive mode $(M + H)^+$ at 317.3 in LC-MS. So the molar mass of the compound **4e** is 316.3 g/mol. Yield—65%, M.P—169–175°C.

1H NMR: ($CDCl_3$, 400 MHz, δ in ppm): 3H, s, 2.04 (Ar-CH₃), 3H, s, 3.94 (Ar-OCH₃), 3H, s, 3.90 (Ar-OCH₃), 1H, s, 6.14 (Ar-H), 2H, m, 2.9 (O=C-CH₂), 1H, dd, 5.69 (>C<H), 1H, m, 7.6 (Ar^1 -H), 1H, m, 7.3 (Ar^1 -H), 1H, m, 7.2 (Ar^1 -H), 1H, m, 7.1 (Ar^1 -H).

^{13}C NMR: ($CDCl_3$, 100 MHz, δ in ppm) 7.74 (Ar-CH₃), 106.41 (Ar-C-CH₃), 161.13 (Ar-C-OCH₃), 88.80 (Ar-C-H), 160.62 (Ar-C-OCH₃), 105.97 (Ar-C-C=O), 163.56 (Ar-C-O), 56.10 (Ar-OCH₃), 55.64 (Ar-O-CH₃), 189.50 (Ar-C=O), 115.5 (Ar^1 -C<), 126.8 (Ar^1 -C-H), 129.8 (Ar^1 -C-H), 160.96, (Ar^1 -C-F), 127.15 (Ar^1 -C-H), 124.4 (Ar^1 -C-H), 44.73 (O=C-CH₂), 73.14 (>C-H).

Compound 4f. It is having the molecular formula $C_{18}H_{17}O_4Br$ which showed molecular ion peak in positive mode $(M + H)^+$ at 378.1 in LC-MS. So the molar mass of the compound **4f** is 377 g/mol. Yield—60%, M.P—166–170°C.

1H NMR: ($CDCl_3$, 400 MHz, δ in ppm): 3H, s, 2.04 (Ar-CH₃), 3H, s, 3.93 (Ar-OCH₃), 3H, s, 3.90 (Ar-OCH₃), 1H, s, 6.12 (Ar-H), 2H, m, 2.8 (O=C-CH₂), 1H, dd, 5.3 (>C<H), 2H, d, 7.5 (Ar^1 -H), 2H, d, 7.3 (Ar^1 -H).

^{13}C NMR: ($CDCl_3$, 100 MHz, δ in ppm): 7.78 (Ar-CH₃), 106.31 (Ar-C-CH₃), 160.84 (Ar-C-OCH₃), 88.73 (Ar-C-H), 160.56 (Ar-C-OCH₃), 105.89 (Ar-C-C=O), 163.64 (Ar-C-O), 56.05 (Ar-OCH₃), 55.63 (Ar-O-CH₃), 189.33 (Ar-C=O), 131.84 (Ar^1 -C<), 122.24 (Ar^1 -C-H), 127.51 (Ar^1 -C-H), 138.41 (Ar^1 -C-Br), 127.51 (Ar^1 -C-H), 122.24 (Ar^1 -C-H), 45.50 (O=C-CH₂), 77.77 (>C-H).

Compound 4g. It is having the molecular formula $C_{20}H_{22}O_6$ which showed molecular ion peak in positive mode $(M + Na)^+$ at 381.3 in LC-MS. So the molar mass of the compound **4g** is 359.3 g/mol. Yield—75%, M.P—179–182°C.

1H NMR (400MHz, δ in ppm, $CDCl_3$): 1H,s, 6.13 (Ar-C-H), 3H,s, 2.06 (Ar-CH₃), 2H,s,7.0 (Ar^1 -C-H), 1H,d, 6.98 (Ar^1 -C-H), 1H, dd, 5.3 (Ar^1 -O-C-H), 3H,s,3.93 (Ar-OCH₃), 9H,s,3.90 (Ar-OCH₃), 2 (Ar^1 -OCH₃), 1H, dd and 1H, dd, 2.97 and 2.86 (—O=C-CH₂—).

^{13}C NMR: ($CDCl_3$, 100 MHz, δ in ppm) 7.83 (Ar-CH₃), 106.29 (Ar-C-CH₃), 161.17 (Ar-C-OCH₃), 88.59 (Ar-C-H), 160.52 (Ar-C-OCH₃), 106.29 (Ar-C-C=O), 160.52

TABLE 2: Antioxidant and cytotoxic activity of chalcones (Compounds **3a-h**).

Test name	Superoxide inhibitory activity			DPPH inhibitory activity			Brine shrimp Lethality test
	Dose at ($\mu\text{g/mL}$)	% of inhibition	IC ₅₀	Dose at ($\mu\text{g/mL}$)	% of inhibition	IC ₅₀	
Compound- 3a	50	4.58	93.89	50	8.36	85.74	46.56
	75	28.92		75	32.12		
	100	57.03		100	69.94		
Compound- 3b	50	7.35	90.95	50	20.73	78.49	56.45
	75	25.33		75	47.27		
	100	63.56		100	71.52		
Compound- 3c	50	18.63	86.58	50	23.15	75.42	63.29
	75	36.76		75	50.06		
	100	69.44		100	75.52		
Compound- 3d	50	25.82	85.52	50	20.61	77.29	32.45
	100	45.42		75	47.64		
	150	79.25		100	74.42		
Compound- 3e	25	30.88	83.27	50	28.97	74.90	28.34
	50	47.06		75	49.94		
	75	78.43		100	71.52		
Compound- 3f	50	31.05	80.15	50	20.48	91.14	35.26
	75	61.76		75	36.97		
	100	76.63		100	57.09		
Compound- 3g	50	12.75	86.58	50	23.15	75.42	38.56
	75	33.01		75	50.06		
	100	66.83		100	75.52		
Compound- 3h	25	6.05	85.52	50	20.61	77.29	25.36
	50	37.09		75	47.64		
	75	56.37		100	74.42		
Vit-C	2.5	34.49	3.98	2.5	34.49	3.98	Phodophyllotoxin 3.69
	5	62.71		5	62.71		
	10	102.27		10	102.27		

(Ar-C-O), 56.07 (Ar-OCH₃), 55.99 (Ar-O-CH₃), 190.03 (Ar-C=O), 131.93 (Ar¹-C<), 109.40 (Ar¹-C-H), 118.38 (Ar¹-C-H), 149.15 (Ar¹-C-OCH₃), 149.23 (Ar¹-C-OCH₃), 111.27 (Ar¹-C-H), 55.97 (Ar¹-OCH₃), 55.63 (Ar¹-OCH₃), 45.59 (O=C-CH₂), 78.29 (>C-H).

Compound 4h. It is having the molecular formula C₁₈H₁₆O₄F₂ which showed molecular ion peak in positive mode (M + Na)⁺ at 357.2 in LC-MS. So the molar mass of the compound-**4h** is 334 g/mol. Yield—55%, M.P—176–180°C.

¹H NMR (400MHz, δ in ppm, CDCl₃): 1H, t, 7.34 (Ar¹-H), 2H, m, 7.2 (Ar¹-H), 1H, s, 6.1 (Ar-C-H), 1H, dd, 5.3 (>C<H), 3H, s, 3.9 (Ar-OCH₃), 3H, s, 3.9 (Ar-OCH₃), 2H, m, 2.8 (O=C-CH₂), 3H, s, 2.05 (Ar-CH₃).

¹³C NMR: (CDCl₃, 100 MHz, δ in ppm) 7.81 (Ar-CH₃), 106.32 (Ar-C-CH₃), 160.64 (Ar-C-OCH₃), 88.32 (Ar-C-H), 163.71 (Ar-C-OCH₃), 105.85 (Ar-C-C=O), 160.59 (Ar-C-O), 56.08 (Ar-OCH₃), 55.66 (Ar-O-CH₃), 189.02 (Ar-C=O), 136.44 (Ar¹-C<), 115.21 (Ar¹-C-H), 121.13 (Ar¹-C-F), 121.84 (Ar¹-C-F), 115.02 (Ar¹-C-H), 117.6 (Ar¹-C-H), 45.54 (O=C-CH₂), 77.32 (>C-H).

Compound 5. It is having the molecular formula C₁₆H₁₄O₅ which showed molecular ion peak in negative mode (M - H)⁻ at 285.1 in LC-MS. So the molar mass of the compound **5** is 286 g/mol.

¹H NMR: (CDCl₃, 400 MHz, δ in ppm): 1H, s, 9.65 (Ar-OH), 1H, s, 9.407 (Ar-OH), 1H, s, 9.339 (Ar¹-OH), 1H, t, 7.030 (Ar¹-OH), 1H, d, 6.822 (Ar¹-OH), 1H, t, 6.622 (Ar¹-H), 1H, t, 6.44 (Ar¹-H), 1H, s, 6.260 (Ar-H), 1H, d, 4.636 (O-C-H), 2H, dd, 3.047 (O=C-CH₂), 3H, s, 1.984 (Ar-CH₃).

¹³C NMR: (CDCl₃, 400 MHz, δ in ppm) 7.81 (Ar-CH₃), 106.36 (Ar-C-CH₃), 163.56 (Ar-C-OH), 88.64 (Ar-C-H), 161.52 (Ar-C-OH), 105.98 (Ar-C-C=O), 160.52 (Ar-C-O), 189.83 (Ar-C=O), 129.73 (Ar¹-C<), δ at 159.90 (Ar¹-C-OH), 113.63 (Ar¹-C-H), 140.99 (Ar¹-C-H), 111.58 (Ar¹-C-H), 118.01 (Ar¹-C-H), 44.65 (O=C-CH₂), δ at 74.23 (>C-H).

3.2. Bioactivity Studies

3.2.1. Antituberculosis. Compounds **3a-h**, **4a-h**, and **5** (pisonivanone) were screened for antituberculosis on Myco

TABLE 3: Antioxidant and cytotoxic activity of Flavanones (Compounds **4a–h**).

Test name	Superoxide inhibitory activity			DPPH inhibitory activity			Brine shrimp Lethality test ED50
	Dose at ($\mu\text{g/mL}$)	% of inhibition	IC50	Dose at ($\mu\text{g/mL}$)	% of inhibition	IC50	
Compound- 4a	10	13.89	44.99	10	11.27	48.77	34.11
	25	37.58		25	35.76		
	50	54.41		50	58.18		
Compound- 4b	10	26.58	29.18	10	14.06	36.35	22.55
	25	48.67		25	40.73		
	50	72.00		50	65.21		
Compound- 4c	10	24.02	33.00	10	25.61	29.24	31.22
	25	43.30		25	42.68		
	50	67.65		50	78.15		
Compound- 4d	10	20.75	32.68	10	26.20	25.81	27.33
	25	47.55		25	57.34		
	50	67.16		50	77.29		
Compound- 4e	10	28.27	28.77	10	9.70	42.39	41.82
	25	49.02		25	37.94		
	50	71.41		50	55.76		
Compound- 4f	10	10.23	39.03	10	18.30	38.08	52.15
	25	32.81		25	39.27		
	50	64.06		50	61.45		
Compound- 4g	10	17.65	36.24	10	17.82	32.28	28.93
	25	42.16		25	46.79		
	50	63.73		50	70.30		
Compound- 4h	10	17.34	31.95	10	16.48	33.28	42.56
	25	41.35		25	43.64		
	50	75.64		50	70.30		
Compound- 5	5	12.42	25.32	10	31.53	20.14	14.35
	10	46.41		25	64.50		
	25	66.18		50	86.76		
Vit-C	2.5	34.49	3.98	2.5	34.49	3.98	Phodophyllotoxin 3.69
	5	62.71		5	62.71		
	10	102.27		10	102.27		

bacterium tuberculosis in LJ medium by using convention method [16] as follows.

Drug Free media (Lowenstein Jensen media) base was prepared according to manufacturer's instructions (HiMedia, Merck). The culture media was prepared by weighing 18.65 grams of the LJ medium base and dissolved in 300 mL of distilled water. Six mL glycerol (reagent grade) was added. The solution was autoclaved at 121°C for 30 minutes and cooled. 500 mL of homogenized whole egg was added and mixed. Then 6–8 mL of the medium was dispensed into a 28 × 110 mm screw capped Mac Cartney culture tubes (Universal containers). These culture tubes were inspissated at 85°C for 55 minutes. For sterility check, prepared culture media were incubated at 37°C for 48 hours and were kept in the refrigerator for storage when no bacterial contaminants were detected. All tubes were tightly capped to prevent evaporation during storage.

Drug containing media was also prepared as above. Drug containing media was prepared as given in Table 1. Stock solution was prepared by dissolving the 10 mg of drug (Compounds **3a–h**, **4a–h**, and Compound **5**) in 5 mL of DMF. Final concentration of stock solution is considered as 2000 mg/L.

3.2.2. Drug Susceptibility Test. MTB isolate (preferably H37 Rv) were tested for drug susceptibility using the absolute concentration method on egg-based LJ medium, using a standardized inoculums (4 mg/4 mL) grown on drug free media along with media containing graded concentrations of the drug(s). All the test cultures are kept incubator at 37°C for 4–6 weeks.

The susceptibility test of antitubercular drugs was examined using five concentrations of Compounds **3a–h**, **4a–h**, and **5** (12.5, 25, 50, 100, and 200 mg/L).

Resistance is expressed in terms of the lowest concentration of the drug that inhibits growth, that is, minimal inhibitory concentration (MIC).

3.3. Antioxidant Activity.

Superoxide Scavenging Method. Superoxide scavenging activity of the compounds **3a-h**, **4a-h**, **5a-e**, and **6a-e** was determined by the method [17] modified by [18] Kutlan et al., which depends on the light induced superoxide generation by riboflavin and the corresponding reduction of NBT. The assay mixture contained different concentration of the test substances and EDTA (6 mM containing 3 μ g NaCN), NBT (50 μ M) riboflavin (2 μ M), and phosphate buffer 58 mM, pH 7.8 in a total volume of 3 mL. The tubes received uniform illumination for 15 min and thereafter optical density was measured at 560 nm. Consider the following:

$$\% \text{ inhibition} = \left[\frac{(\text{control} - \text{sample})}{\text{control}} \right] \times 100. \quad (1)$$

An IC_{50} value was determined as the concentration that elicited the half maximal response.

Statistical Analysis. The data were analyzed by (single factor) for multiple groups and the significance level was chosen as $P < 0.05$. Data is expressed as the mean (+) or (–)-SEM with a minimum of three experiments performed per each variable.

3.4. DPPH Free Radical Scavenging Activity. DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity of the synthesised compounds **3a-h**, **4a-h**, and **5** was determined by the method [19] of Lamaison et al., which depends on scavenging of colored free radical (DPPH) in methanol solution by the test drugs. The reaction mixture contains DPPH and test drug in a final concentration of 3 mL. Absorption of DPPH at its adsorption maximum 516 nm is inversely proportional to the concentration of the scavenger (test drug). The activity was expressed as inhibitory concentration 50 (IC_{50}), that is, the concentration of the test solution required to give 50% reduction in absorbance of the test solution as compared to that of blank solution. Consider

$$IC_{50} = \left[\frac{(\text{OD of control} - (\text{OD of test} - \text{OD test blank}))}{\text{OD of control}} \right] \times 100. \quad (2)$$

The results obtained by both methods were given in Tables 2 and 3.

3.5. Cytotoxic Activity. Cytotoxic activity of the synthesised compounds **3a-h**, **4a-h**, and **5** were determined by using a known method [20, 21]. 5 mL of brine solution is taken into each test tube. Test substances were added to the tube according to their concentrations. The solutions were thoroughly mixed with the help of the cyclomixer. Then 10 shrimps were added to each test tube. Suitable conditions like temperature should be maintained for proper results. Finally by observing

the lethality rate of the shrimps after 24 hours the ED_{50} value is calculated. The ED_{50} using probed analysis at 95% confidence limits from observed data. Replicas maintained to get accurate results (Tables 2 and 3).

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