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SYNTHESIS AND BIOLOGICAL SCREENING OF ANALOGS OF ARYL TETRALONE

Chaitramallu M[1], Devaraju Kesagodu[1], Dakshayini Chandrashekarachar[2]
[1]*Assistant Professor, Department of Chemistry, Yuvaraja's College, Mysuru.*
[2]*Department of Chemistry, Government College for Women, Mandya.*

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ABSTRACT

The aryl tetralone as potential antimitotic agents were synthesized in four step reactions using Grignard reagent. The first step is the synthesis of trimethoxy phenyl naphthol (2a-f) by the reaction of substituted tetralone with 3, 4, 5-trimethoxy 1-bromobenzene in magnesium metal using tetrahydrofuran as a solvent. The resulted phenyl naphthol was hydrogenated to give phenyl tetralin 3(a-f). The substituted phenyl tetralone were prepared by the oxidation of trimethoxy phenyl tetralin 4(a-f). The structures of the synthesized compounds were confirmed by spectral and elemental analysis data. The synthesized compounds were also screened for their antidiabetic activity. It is noteworthy that compounds 4d possessed excellent antidiabetic activity, 4b and 4c showed considerable activity, and remaining 4a possessed least activity. Compound 4d was immense to show high antibacterial and antifungal activity against all bacterial and fungal strains compared with standard chloramphenicol and nystatin, respectively.

Corresponding author
Dr. Devaraju
Assistant professor
Department of Chemistry,
University of Mysore, yuvaraja's college,
Mysore, 570006, Karnataka, India.
passion49432005@gmail.com

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INTRODUCTION

The synthesis of aryl tetralone has attracted the attention of many research groups because of their interesting pharmacological activities [1-2]. An efficient, short step and high yielding approach of these tetralones will be of great utility for large scale synthesis of several bioactive compounds for their complete preclinical and clinical evaluations. Several, approaches have been developed for the synthesis of tetralone [2-3]. In some of these methods we have observed (i) long reaction sequences affording low to moderate yield, (ii) difficult experimental procedures which require skilled experimentalists to reproduce the results, (iii) the use of expensive reagents (rhodium, triphenylphosphonium chloride, ionic liquids etc) and (iv) the use of reagents (diazoketone,ethylene gas, βketosulfoxide etc) which are injurious for health [4-7]. Analyzing some of these draw backs and considering our interest on the synthesis of α and β-substituted tetralones. We believe that still a more efficient and facile synthesis can be developed for tetralones 4a-f which have proved to be potential intermediate for the synthesis of several bioactive compounds. We have sought a facile synthesis of the tetralones 4a-f by a single route instead of independent approach for each of these tetralones. To the best of our knowledge this is the first example of the synthesis of the tetralones 4a-f by this route and the present paper documents our results

EXPERIMENTAL

MATERIALS AND METHODS

All reagents and chemicals were purchased from Merck. They were used without further purification. Melting points were taken in open capillary tubes and are uncorrected. Thin-layer chromatography (TLC) is performed with E. Merck precoated silica gel plates (60F-254) with iodine as a developing agent. Acme, India silica gel, 60–120 mesh is used for column chromatography. IR spectra in KBr were recorded on Perkin-Elmer model 683 spectrometers. ^{1}H NMR (400 MHz), and ^{13}C NMR (100 MHz) spectra were recorded using tetramethyl silane (TMS) as an internal reference on Bruker spectrometer, Elemental analysis were performed on a Perkin-Elmer 2400. Mass spectra were obtained by Water-Q-TOF ultima spectrometer. Micro analytical data were obtained by elemental-Vario EL-III.The syntheses of our target compounds are described in Scheme 1

Synthesis

General procedure for the synthesize of phenyl tetralin naphthol analogues 2(a-f)

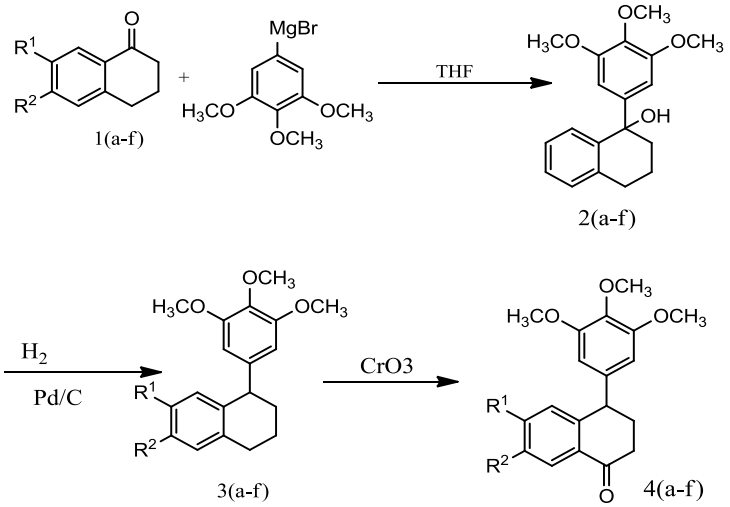
A Grignard reaction was prepared in an oven dried three necked flask outfitted with a reflux condenser, dropping funnel and magnetic stirrer. Approximately 1/4th of a 10mmol aliquot of 1-bromo-3, 4, 5-trimethoxybenzene in 5ml of anhydrous THF was added to a mixture of magnesium turnings (10mmol) in 2.5ml of anhydrous THF with a small piece of iodine. As soon as the reaction mixture becomes colourless the remaining 1-bromo-3, 4, 5-trimethoxybenzene solution was added drop wise to the solution under mild reflux stirring was then continued for1h at room temperature. A solution of 3, 4, 5-trimethoxyphenyl magnesium bromide (10mmol) was added slowly to the substituted tetralone 1 (a-f) 8.35mmol in 2.5ml anhydrous THF solution at 0^{0}C. After complete addition, the solution was allowed to stir at room temperature for another 20min, a saturated ammonium chloride(10ml) solution was added to hydrolyze the adduct at 0^{0}C and the mixture was stirred for 10min. the phases was separated and the aqueous layer was extracted with ether (10ml in three portion) the combined organic layer were stirred washed with brine solution and dried over $MgSO_4$ and filtered the filtrate was concentrated in vacuum and the residue was purified by recrystallization.

General procedure for the synthesize of phenyl tetraline analogues 3(a-f)

Substituted tetralin alcohol 1(a-f) (5g, 0.0196 mole) was subjected to hydrogenation over 10% Pd-C in ethanol acetic acid (35:1.25v/v) mixture. The catalyst was filtered off and filtrate was distilled to remove ethanol and the residue was extracted with ether. The ether extract was washed with water and dried to give the crude product which was purified by column chromatography over silica gel using petroleum ether.

General procedure for the synthesis of substituted phenyl tetralone 4(a-f)

To a stirred solution of compound 2(a-f) in acetic acid (14ml), the propionic acid (5ml) was added with stirring at 0^{0}C followed by chromium trioxide (3.0g) in water was added. The reaction mixture was stirred at 0-5^{0}C for 7hr. After completion of the reaction, the reaction mixture was poured into ice-water and extracted with ether. The ether extract was washed with water, sodium carbonate solution and again with water, dried over magnesium sulfate and recrystallized using ethanol to give pure compound.



Scheme 1: protocol for the synthesis of aryl tetralones.

Where	R_1	R_2
a	OCH_3	OCH_3
b	H	OH
c	H	CH_3
d	H	Cl
e	H	H
f	H	OCH_3

Biological assays

Anti microbial activity

Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria (*Bacilus subtilius, Streptococcus*) and Gram-negative bacteria (*Escherichia coli*, *Proteus*) in DMF by disc diffusion method on nutrient agar medium. The sterile medium (nutrient agar medium,15ml) in each petriplate was uniformly smeared with cultures of Gram-positive and Gram-negative bacteria. Sterile discs of 10mm diameter (Hi-Media) was placed in the petriplates, to which different concentrations of drug (20µg,40,80,100µg/disc) of the different synthesized compounds were added. Gentamycin was used as positive control for comparison. For each treatment, three replicates were maintained. The plates were incubated at 37^0C for 24h and the zone of inhibition was determined [8-9]**.**

Antioxidant assays

DPPH radical scavenging assay

DPPH radical reacts with an Anti-oxidant compound that can donate hydrogen and get reduced. DPPH, when acted upon by an Anti-oxidant, is converted into diphenyl picryl hydrazine. This can be identified by the conversion of purple to light yellow.

DPPH radical scavenging activity was done by the method of Channasammi Gandhimathi et al., [10]. 1ml of DPPH solution (0.1Mm, in 95% ethanol) was mixed with different concentration of compounds, shaken & incubated for 20min at room temperature & absorbance was read at 517nm against a blank. The radical scavenging activity was measured as the decrease in the absorbance of DPPH & calculated using the following equation:

Scavenging effect (%) = A control (540nm) - A sample (540nm)/A control (540nm) ×100

Nitric oxide radical scavenging assay

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside, in aqueous solution at physiological pH, spontaneously generates nitric-oxide, which in turn reacts with oxygen to produce nitric ions that can be estimated by the Griess reagent spectrophotometrically at 540nm[11].

Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5mM) in phosphate buffer saline was mixed with the compounds & incubated at 25^oC for 120minutes. The samples from the above were reacted with Griess reagent. The absorbance of the chromophore form during the diazotization of nitrate with sulphonyl amide & subsequent coupling with naphthyl ethylene diamine was read at 540nm & refer to the absorbance standard solutions of BHT, treated in the same way with Griess reagent. The radical scavenging activity was measured using the equation described below:

Scavenging effect (%) = A control (540nm) - A sample (540nm) /A control (540nm) ×100

RESULTS AND DISCUSSION

Chemistry

The new tetralone analogues of podophyllotoxin were synthesized via Grignard reaction (Scheme 1). Substituted aryl tetralones 1(a-f) were subjected to Grignard reagent of 3, 4, 5-trimethoxymagnesium bromide in THF solvent. In all the cases reaction took place at ketone position to form alcohol, which was recrystallised by ethanol as shown in the structure 2(a-f), the synthesized compounds were confirmed by IR, ^{1}HNMR and mass spectra. As a specific example, IR spectra of compounds 2a showed the C=C stretching frequency in the range 1549cm^{-1} and stretching vibration frequency of C-OH in the region 3650-3550cm^{-1}. ^{1}H NMR of compound 2a showed the absence of ketone group indicating peak at 2.87-2.84ppm and the presence of OH group at 3.65ppm and mass spectra confirms the peak (M^{+}) at 374.14

The phenyl tetralin (3a-f) were prepared in good yields by the hydrogenation of phenyl naphthol (2a-f) with Pd/C catalyst. These phenyl tetralin analogues of 3a showed IR stretching absorptions for C-C stretching bond appeared at 1269 cm^{-1} and for the C_6H_5-CH in 3183–2975 cm^{-1}. The ^{1}HNMR spectra of compounds 3a was consistent with the structure assigned and showed the ^{1}HNMR signals, a triplet at 2.87 - 2.84 assigned to the CH_2 protons and the absence of OH group at 3.65ppm confirms the structure.

Tetralones (4a-f) were prepared were synthesized by oxidation using chromium trioxide. This readily gives up proton to form tetralones. In its IR spectra appeared absorption bands in the range 2923-2853cm^{-1} and 1683-1675 cm^{-1} corresponds respectively to C-H and C=O stretching frequencies and ^{1}H NMR of the ring B protons appears in the range 2.65-2.18 ppm. They are the key intermediates for the synthesis of the novel nitrogen containing analogues of podophyllotoxin.

6, 7-dimethoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one 4a
Color: dark brown solid. Yield: 75.18%.
IR: 3128–2939 (Ar-CH), 1697 (C=O);
^{1}H NMR: 7.58–7.05 (4 H, m, Ar-H), 6.52(2 H, s, Ar-H), 5.35(1H, d, OH), 4.04 (1 H, t, *J* = 4.7, CH), 3.83 (15 H, s, OCH_3), 2.66–2.25 (4 H, t, *J* = 6.4, CH_2);
^{13}C NMR: 198.0, 165.9, 141.7, 140.1, 130.5, 129.5, 128.5, 126.5, 111.9, 104.7, 55.7, 45.6, 37.4, 31.4, 14.5;
MS, *m*/*z*: 372.15 (*M*+). Anal. Calcd. For $C_{21}H_{24}O_6$: C, 67.73; H, 6.50 O, 25.78 Found: C, 67.76; H, 6.51 O, 25.79 %.

6-hydroxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one 4b
Color: brown semi solid. Yield: 82.18%.
IR: 3128–2939 (Ar-CH), 1667 (C=O);
^{1}H NMR: 8.19–6.60 (3 H, m, Ar-H), 6.52(2 H, s, Ar-H), 5.35(1H, d, OH), 4.04 (1 H, t, *J* = 4.7, CH), 3.83 (9 H, s, OCH_3), 2.60–1.25 (4 H, t, *J* = 6.4, CH_2);
^{13}C NMR: 198.0, 161.9, 145.7, 141.9, 130.9, 129.5, 128.5, 126.5, 111.9, 55.7, 45.6, 37.4, 31.4, 14.5;
MS, *m*/*z*: 238.15 (*M*+). Anal. Calcd. For $C_{21}H_{24}O_6$: C, 69.53; H, 6.15 O, 24.36 Found: C, 69.56; H, 6.14 O, 24.39 %.

6-methyl-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one 4c
Color: dark brown gummy solid. Yield: 81.94%.
IR: 3125–2938 (Ar-CH), 1695 (C=O);
^{1}H NMR: 7.80–7.13 (5 H, m, Ar-H), 6.52(2 H, s, Ar-H), 4.06 (1 H, t, *J* = 4.8, CH), 3.80(9H, s, OCH_3), 2.65–2.28 (4 H, t, *J* = 6.5, CH_2), 2.34 (3 H, s, CH_3); ^{13}C NMR: 198.1, 153.4, 143.3, 140.4, 137.3, 136.7, 131.4, 128.0, 126.4, 125.2, 106.7, 60.9, 56.3, 45.9, 37.5, 31.6, 21.6;
MS, *m*/*z*: 326.14 (*M*+). Anal. Calcd. For $C_{20}H_{22}O_4$: C, 73.60; H, 6.79 O, 19.61 Found: C, 73.61; H, 6.75 O, 19.69 %.

6-chloro-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one 4d
Color: dark brown gummy solid. Yield: 88.94%.
IR: 3120–2938 (Ar-CH), 1685 (C=O);
^{1}H NMR: 7.86–7.39 (5 H, m, Ar-H), 6.52(2 H, s, Ar-H), 4.06 (1 H, t, *J* = 4.8, CH), 3.88(9H, s, OCH_3), 2.65–2.58 (2 H, q, *J* = 6.5, CH_2), 2.34-1.98 (2 H, q, CH_2);
^{13}C NMR: 190.8, 152.4, 141.9, 139.3, 137.7, 136.4, 132.1, 127.9, 126.2, 106.9, 60.9, 56.3, 45.1, 37.5, 31.6, 21.6;
MS, *m*/*z*: 346.14 (*M*+). Anal. Calcd. For $C_{20}H_{22}O_4$: C, 65.80; H, 5.52; Cl 10.22; O, 18.45 Found: C, 65.81; H, 5.55; Cl 10.20; O, 18.49 %.

4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one 4e
Color: dark brown gummy solid. Yield: 88.81%.
IR: 3128–2935 (Ar-CH), 1691 (C=O);
^{1}H NMR: 7.83–7.33 (6 H, m, Ar-H), 6.52(2 H, s, Ar-H), 4.02 (1 H, t, *J* = 4.6, CH), 3.83(9H, s, OCH_3), 2.64–2.26 (4 H, t, *J* = 6.3, CH_2).
^{13}C NMR: 198.0, 153.4, 140.6, 140.5, 137.3, 136.7, 133.6, 128.1, 126.1, 106.1, 60.8, 56.5, 45.6, 37.6, 31.4, 14.9;
MS, *m*/*z*: 312.07 (*M*+). Anal. Calcd. For $C_{19}H_{20}O_4$: C, 73.06; H, 6.45 O, 20.49. Found: C, 73.07; H, 6.43, O, 20.50%.

6-methoxy-4-(3, 4, 5-trimethoxyphenyl)-3, 4-dihydronaphthalen-1(2H)-one 4f
Color: dark brown gummy solid. Yield: 85.18%.
IR: 3128–2939 (Ar-CH), 1697 (C=O);
^{1}H NMR: 8.28–6.85 (5 H, m, Ar-H), 6.52(2 H, s, Ar-H), 4.04 (1 H, t, *J* = 4.7, CH), 3.80(12H, s, OCH_3), 2.66–1.95 (4 H, t, *J* = 6.4, CH_2);
^{13}C NMR: 198.0, 165.9, 153.6, 141.5, 137.5, 136.7, 130.5, 126.5, 111.9, 106.7, 60.9, 56.1, 55.8, 45.9, 37.4, 31.4;
MS, *m*/*z*: 342.15 (*M*+). Anal. Calcd. For $C_{20}H_{22}O_5$: C, 70.16; H, 6.49 O, 23.36 Found: C, 70.17; H, 6.45 O, 23.39 %.

Biological assay
Anti-Microbial activities

The synthesized compound were analysed for antimicrobial assay, Compounds (4a, 4b, 4c and 4d) were tested in vitro for their antimicrobial activity against 2 Gram-positive, 2 Gram-negative bacteria & a yeast type fungi C.*albican* strains. Commercial antibiotics such as Gentamycin & Flucanozole were used as reference drugs. The results were compared with reference drugs & depicted in the above table. The table1 reveals that 4a, 4b, and 4d is potent of all the compounds which were under study with the MIC values ranging from 1.2µg to 7µg. Compounds 4c was not acting on Gram-positive bacteria such as *B.subtilis*. Compared to the reference compounds the activity of the tetralone derivatives is significant.

Table 1:-Invitro Anti-Microbial activities of synthesized compounds.

Compounds	Minimum inbitory concentration (µg)				
	S.aureus	***B.subtilis***	***E.coli***	***Proteus***	***C.albicans***
4a	3	7	4	3	1.4
4b	1.3	3.5	2.4	3	1.3
4c	3.5	-	3.5	4	55
4d	1.2	3.3	2.3	2	1.2
Gentamycin	0.5	0.72	1	1.3	-
Flucanozole	-	-	-	-	0.75

Antioxidant activities

The synthesized compounds were analyzed for antioxidant activity by DPPH radical scavenging activity and nitric oxide radical activity.

DPPH radical scavenging assay

All the compounds of tetralin derivatives showed significant scavenging activity of the DPPH radicals compared to the reference compound BHT. Compounds **4a** and **4c** showed potent DPPH scavenging activity with an IC_{50} value of 16.89 µg/mL, 17.24 µg/mL 18.12 µg/mL, 18.15 µg/mL, 18.75µg/mL. Compound 5b showed less DPPH scavenging activity.

Table 2:-Invitro DPPH radical scavenging assay of synthesized compounds.

Compounds	IC_{50} µg/ml
4a	17.24
4b	35.62
4c	15.25
4d	25.83
BHT	37.4

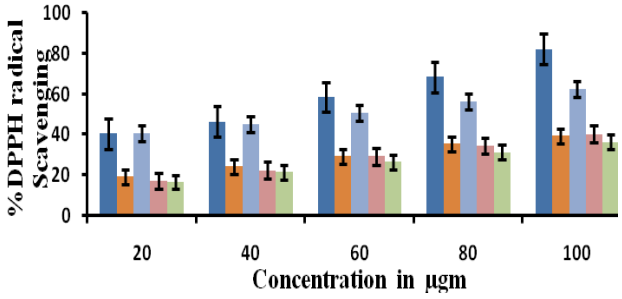


Figure 1:- DPPH radical scavenging assay of synthesized compounds.

Nitric oxide radical scavenging assay

All the tetralone derivatives showed the scavenging of nitric oxide radicals, scavenging is not significant compared to the reference compound BHT 6.4µg/mL.

Table 3:-Invitro nitric oxide radical scavenging assay of synthesized compounds.

Compounds	IC_{50} (µg/ml)
4a	30.62
4b	35
4c	30.62
4d	36
4e	31.25
BHT	6.4

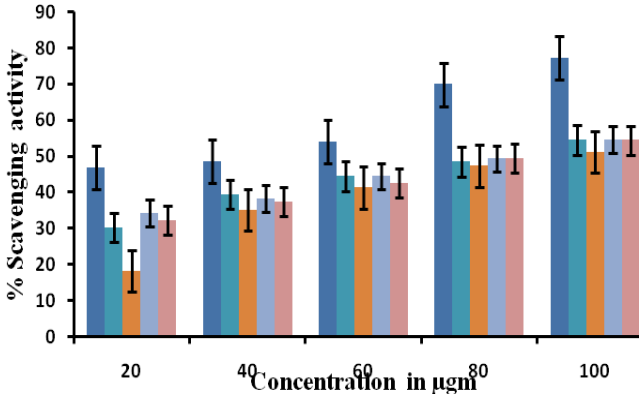


Figure 2: - Nitric oxide radical scavenging assay.

CONCLUSION

The aryl tetralone analogues (4a-f) were prepared in good yield which are very essential for the synthesis of podophyllotoxin and their analogues. They showed good anti-microbial, anti-oxidant and anti-inflammatory effects for the treatment of various microbial infections.

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