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Synthesis of a novel plant growth promoter from gallic acid☆

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Abstract—Gallic acid has been modified to naphthophenone derivatives with esterified fatty acid side chain. Compound **12**, an ethyl crotonate ester of naphthophenone derivative has shown potent auxin like growth promoter activity. This is the first example of naphthophenone derivatives with plant growth promoting activity.

1. Introduction

Gallic acid (**1**) and its related compounds are widely distributed in plants.[2,3] It has been reported to possess anticarcinogenic, antioxidative, antimutagenic, anti-allergic and anti-inflammatory activities.[4] Gallic acid has been a building block of choice for different pharmaceutical leads due to the presence of this moiety in several bioactive natural products.[5] Hence, numerous derivatisations have been done and are reported as anticancer agents,[6] HIV-1 Integrase[7] and HIV-1RT inhibitors,[8] antioxidants,[9] antimalarials,[10] etc.

While working on structural modification of phytomolecules,[11,12] we report a novel plant growth promoter having potent auxin like growth promoting activity derived from gallic acid.

2. Chemistry

The methodology adopted for the synthesis of above molecules is presented in Scheme 1. Gallic acid was first methylated[13] with dimethyl sulfate in aqueous alkali to give trimethoxy benzoic acid (**2**) in 76% yield. The methylated gallic acid was next esterified with β-naphthol in the presence of DCC/DMAP in dry dichloromethane under refluxing condition to give 3,4,5-trimethoxybenzoic acid naphthalene-2-yl ester (**3**) in 77% yields. Compound **3** then subjected to Fries rearrangement using anhydrous $AlCl_3$ at 120–130 °C under neat conditions gave the desired naphthophenone derivative (**4**) with a yield of 31% along with two byproducts 4-demethylated and 3,4-didemethylated derivatives of starting ester **3**. It is worth mentioning here that the Friedel Crafts reaction of trimethoxy benzoic acid (**2**) with β-naphthol in the presence of various Lewis acids ($AlCl_3$–DCM, $AlCl_3$–CS_2, $AlCl_3$ neat, $ZnCl_2$, BF_3–etherate and $SnCl_4$) was unsuccessful. However, it was successful when PPA was used, but the yield was very poor (less than 5%). Finally, the condensation of naphthophenone derivative **4** with different ethyl bromo ester was affected in the presence of potassium carbonate and dry acetone at room temperature for preparations of compounds **5**, **6**, **10**, **11** and **12** and under refluxing conditions for rest of the compounds (**7**, **8**, **9** and **13**). The desired compounds **5**–**13** were obtained in variable yields, 58–89%.

2.1. Synthesis of 3,4,5-trimethoxy benzoic acid naphthalene-2-yl ester (3)

In a 25 mL round bottom flask a mixture of 3,4,5-trimethoxy benzoic acid (2, 4.0 g, 18.87 mmol), DCC (4.0 g, 19.42 mmol), dry dichloromethane (100 mL) was refluxed for 30 min. To this reaction mixture β-naphthol (2.2 g, 15.28 mmol) was added and refluxing was continued for 3 h. After completion of the reaction, solvent was evaporated off. The residue thus obtained

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☆ See Ref. 1.
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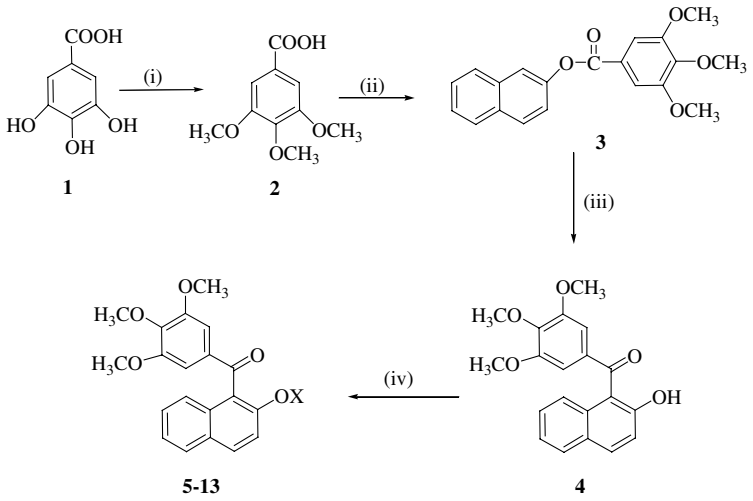


1 2 3 4 5-13

5, X = $-CH_2COOC_2H_5$; **6, X =** $-CH_2.CH_2COOC_2H_5$;
7, X = $-CH_2.CH_2.CH_2COOC_2H_5$; **8, X =** $-CH_2.CH_2.CH_2.CH_2COOC_2H_5$;
9, X = $-CH_2.CH_2.CH_2.CH_2.CH_2COOC_2H_5$; **10, X =** $-CHFCOOC_2H_5$;
11, X = $-CF_2COOC_2H_5$; **12, X =** $-CH_2.CH{=}CH.COOC_2H_5$;
13, X = $-CH_2.CO.COOC_2H_5$.

Scheme 1. Reagents and conditions: (i) Me_2SO_4/KOH, 10–20 °C for 30 min, then reflux for 3 h, 76%; (ii) DCC/DMAP, DCM, 4 h, 71%; (iii) $AlCl_3$ neat, 120–130 °C, 31%; (iv) different ethyl bromo esters, dry acetone, K_2CO_3, 58–89%.

was purified through silica gel column by eluting with hexane–ethyl acetate to get **3** as a white crystalline solid, yield 77%.

2.2. Synthesis of (2-hydroxy-naphthalen-1-yl)-(3,4,5-trimethoxyphenyl)methanone (4)

A mixture of compound **3** (500 mg, 1.48 mmol) and anhyd $AlCl_3$ (1.0 g, 7.49 mmol) was heated at 120–130 °C in an oil bath for 40 min. Then the reaction mixture was cooled and 10 mL dil HCl (1 N) was added to it drop wise. The reaction mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic layer was washed with water, dried over anhyd sodium sulfate and distilled off the solvent to get a yellowish crude oil. It was purified through a silica gel column with a mixture of hexane–ethyl acetate (43:7) as eluent. The pure compound **4** was recrystallised from $CHCl_3$–hexane to get a creamish white crystalline solid, yield 31%.

2.3. General procedure for the synthesis of [(3,4,5-trimethoxy-benzoyl)naphthalene-2-yloxy]-alkanoic/alkenoic acid ethyl esters (5–13)

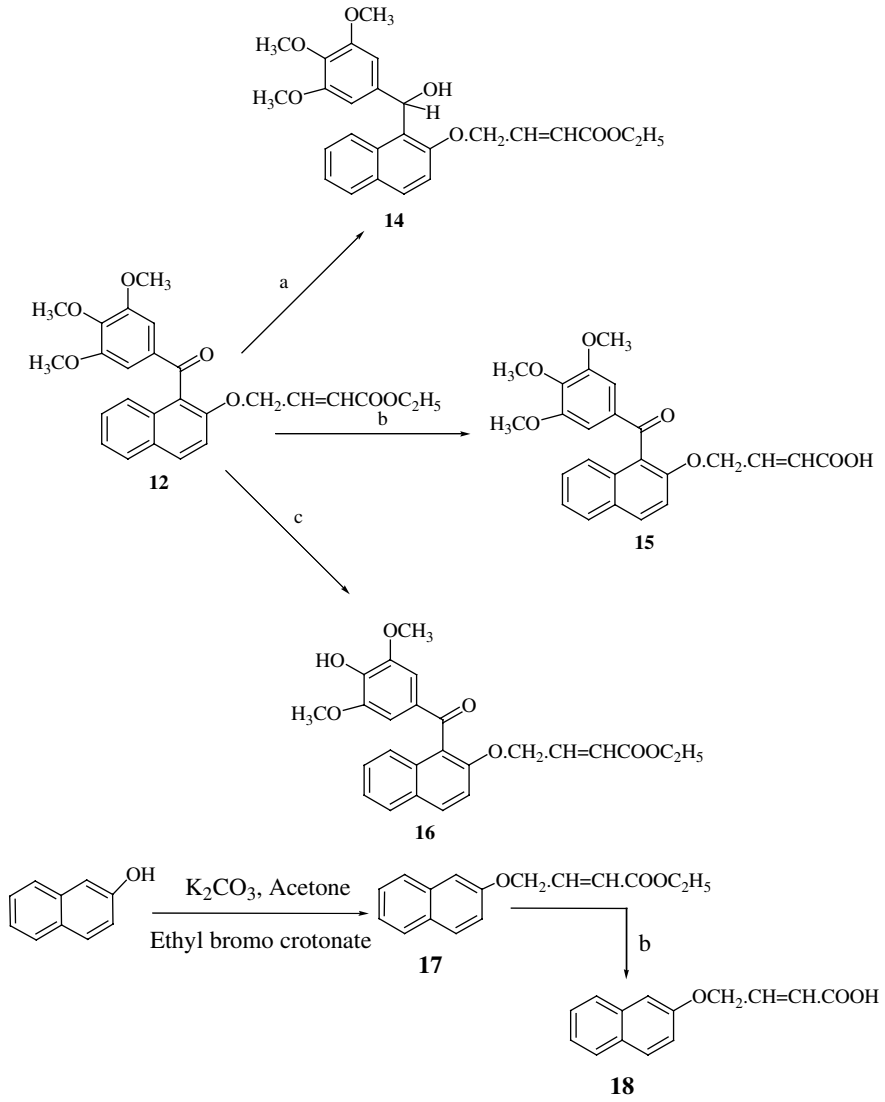
2.3.1. 4-[1-(3,4,5-Trimethoxy-benzoyl)-naphthalen-2-yl-oxy]-but-2-enoic acid ethyl ester (12). A mixture of (2-hydroxy-naphthalen-1-yl)-(3,4,5-trimethoxyphenyl)methanone (**4**, 200 mg, 0.59 mmol) and potassium carbonate (1.0 g, 7.24 mmol) was taken in dry acetone (10 mL) and stirred at room temperature for 2 h. On completion of the reaction the colour of the reaction mixture changed from light yellow to creamish white. The reaction mixture was filtered through sintered funnel, washed with acetone. The solvent was evaporated and the residue was dissolved in ethyl acetate, washed with water (20 mL × 3). The organic layer was dried over anhyd sodium sulfate and distilled off. It was purified through silica gel column using hexane–ethyl acetate as eluent to get **12** as oil,[17] yield 83%.

In order to establish structure–activity relationship (SAR) for the derivatives of **12**, several chemical modifications were tried on it as described in Scheme 2. Reduction of the keto group of **12** into corresponding alcohol **14** resulted in complete loss of activity. When the ester group of **12** was hydrolysed with aqueous methanolic alkali it yielded an inactive acid **15**. Demethylation[14] of the methoxy group *para* to keto group of compound **12** gave inactive product **16**. Replacement of the unsaturated butyrate side chain by saturated chain, for example, compound **17** and **18** resulted in inactive compounds. Thus, it may be concluded that for the expression of activity of **12**, 3,4,5-trimethoxy benzoyl moiety as well as double bond in the side chain are essential.

3. Biological evaluation and discussions

3.1. Growth promoting activity of compound 12 using Bacopa sensor system

For testing the growth promoting effect, all these compounds were dissolved in DMSO and added into the medium at 1.0 μg/mL. Only solvent was used as a control in the experiment for comparison. A fast propagating strain of *Bacopa monnieri* developed[15] as a biosensor system through tissue culture at CIMAP was used in the tests. The MS basal supplemented[16] with test compound was used in the assays. Measured 0.5–1.0 mL medium was poured into 1.5 mL graduated microcentrifuge tubes. Twig cuttings of 2.5 cm were inoculated in 10 replicates for each treatment. These inoculated tubes were put into a half transparent desiccator allowing air



Scheme 2. Reagents and conditions: (a) $NaBH_4$/MeOH, 40–50 °C, 89%; (b) 10% KOH in 10 mL MeOH–H_2O = 3:1, 60 °C, 1 h, 82%; (c) $AlCl_3$–DCM, rt, 62%.

passage through sterile cotton plugs fixed on opening vent. The tubes were placed such that the medium-containing portion of the tubes where roots would be initiating is inserted in to the holes of the stand made from a thermocol sheet. These desiccators were incubated at normal ambient temperature of 25–28 °C with 14 h light and 10 h dark cycle. The root initiation, shoot elongation, callus induction, shoot proliferation and wilting was recorded from day 2 to 14 every 24 h.

3.2. Growth promoting activity of compound 12 using aromatic plant species *Mentha arvensis*

To confirm the growth promoting activity, compound **12** was tested with a medicinal and aromatic plant species *M. arvensis*. The explants used were 0.5 cm long pieces of the second and third internodes of the shoots formed from auxiliary buds and culture. The internode segments were inoculated in MS basal media[16] containing vitamins 100 μg/mL, myo-inositol 3%, w/v, sucrose 1.5% w/v, Agar and different concentrations of auxins and cytokine. Different concentrations of 1-naphthalene acetic acid (NAA) (0.0, 0.2, 2.0 μg/mL) were used in combination with different concentrations of 6-benzyl amino purine (BAP) (0, 5 and 10 μg/mL). On each kind of media 10 replicates of the explants were inoculated into three Petri plates with each plate containing four explants. The compound **12** was serially replaced with each concentration of NAA and BAP individually to observe its growth promoting activity. The experiment was arranged in the form of a completely randomised design (CRD). Cultures were maintained at 25 ± 2 °C and 400–600 lx light intensity with 16 h photoperiod. The response of explants was recorded every 24 h over a four weeks period. Each explant was observed at two week intervals and subcultured on the same fresh medium. The proportional increase in biomass was recorded by taking the fresh weight of the growing tissue during subculturing and dividing the increase with the initial weight. At the end of 12 weeks from inoculation the shoots were separated and individually transferred to MS basal media containing vitamins for rooting.

Table 1. Growth promoting activity of compound **12** using Bacopa sensor system

Test compound **12**	Shoot elongation (cm)	Branching (nos.)	Root elongation (cm)	No. of leaves (nos.)	No. of roots (nos.)	Browning yellowing death
Control	1.0	2.0	0.8	5.0	2+2	Nil
Compound **12**	1.6	2.0	0.8	11.0	2+2	Nil

Table 2. Growth promoting activity of compound **12** with *M. arvensis*

Medium	Concentration of growth regulators in MS basal medium			Observations after number of days		
	IAA[a]	BAP[b]	Compd **12**	**8**	**14**	**22**
A3	2.0	10.0	—	Callusing	Shooting	Shooting
	—	10.0	2.0	Callusing	Shooting	Shooting (single root in one replication)
	2.0	—	10.0	No growth	No growth	No growth
A2	0.2	5.0	—	Callusing	Shooting	Shooting (no roots)
	—	5.0	0.2	Callusing	Shooting	Shooting (no root in any)
	0.2	—	5.0	Less growth	Browning	Death

[a] Indole acetic acid.
[b] 6-Benzyl amino purine.

The rooted plantlets were subsequently transferred to pots in a green house.

The growth promoting activity data of compound **12** using Bacopa sensor system is given in Table 1. Only the compound **12** was found to possess potent growth promoting activity, rest of the compounds were found to be inactive. The growth promoting activity of compound **12** was further confirmed to be auxin like, by another experiment using an aromatic plant *M. arvensis*, given in Table 2.

4. Conclusion

All the compounds except compound **12** were found to be inactive as plant growth promoters. However, compound **12**, possessed potent auxin like growth promoting activity. Compound **12** on modifications to some of its derivatives as described above led to inactive molecules. Thus, it was concluded that the complete naphthophenone moiety is required along with the crotonate ester chain for the activity. Inactivity of compound **5** (acetic acid ester) suggests a possible difference in the SAR of these compounds with that of naphthalene acetic acid. This study suggests that properly functionalised gallic acid derived molecules can also act as plant growth promoters.

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17. *Selected physical data*: compound **3**: yield: 77%, mp = 99–102 °C, ^{1}H NMR ($CDCl_3$): 3.97 (s, 9H, OCH_3), 7.35–7.94 (m, 9H, all aromatic protons), electrospray mass: 360.9 $[M+Na]^+$, 338.9 $[M+H]^+$. Compound **4**: yield: 31%, mp = 138–40 °C, ^{1}H NMR ($CDCl_3$): 3.70 (s, 6H, 3′ and 5′-OCH_3), 3.92 (s, 3H, 4′-OCH_3), 6.90–7.93 (m, 8H, all aromatic protons), 10.85 (s, 1H, phenolic OH); electrospray mass: 360.9 $[M+Na]^+$, 338.9 $[M+H]^+$; elemental analysis for $C_{20}H_{18}O_5$ calcd: C, 70.99; H, 5.36; obsd: C, 71.46; H, 5.18. Compound **12**: yield: 83%, oil, ^{1}H NMR ($CDCl_3$): 1.28 (t, 3H, C*H*$_3$, J = 6.58 Hz), 3.78 (s, 6H, 3′ and 5′-OC*H*$_3$), 3.92 (s, 3H, 4′-OC*H*$_3$), 4.16 (q, 2H, O*CH*$_2$·CH_3, J = 6.7 Hz), 4.76 (br s, 2H, O*CH*$_2$·CH═), 5.76–5.82 (d, 1H, ═*CH*CO, J = 15.8 Hz), 6.89–6.95 (d, 1H, CH_2·*CH*═, J = 15.7 Hz), 7.14–7.96 (m, 8H, all aromatic protons), FAB mass: 451 $[M+H]^+$; elemental analysis for $C_{26}H_{26}O_7$ calcd: C, 69.32; H, 5.82; obsd: C, 68.48; H, 5.34. Compound **14**: yield: 74%, oil, ^{1}H NMR ($CDCl_3$): 3.74 (s, 6H, 3′ and 5′-OC*H*$_3$), 3.89 (s, 3H, 4′-OC*H*$_3$), 4.08 (s, 1H, C*H*OH), 4.21 (s, 2H, OC*H*$_2$), 4.76 (s, 2H, OC*H*$_2$), 7.26–7.89 (m, 8H, all aromatic protons); electrospray mass: 475 $[M+Na]^+$, elemental analysis for $C_{26}H_{28}O_7$ calcd: C, 69.01; H, 6.24; obsd: C, 68.86; H, 6.48. Compound **15**: yield: 69%, mp = 132–35 °C, ^{1}H NMR ($CDCl_3$): 3.87 (s, 6H, 3′ and 5′-OC*H*$_3$), 4.08 (s, 3H, 4′-OC*H*$_3$), 6.60–6.65 (d, 1H, ═C*H*·COOH, J = 15.6 Hz), 6.72 (s, 2H, OC*H*$_2$), 7.53–7.58 (d, 1H, C*H*═, J = 15.6 Hz), 7.26–7.96 (m, 8H, all aromatic protons); electrospray mass: 445 $[M+Na]^+$, elemental analysis for $C_{24}H_{22}O_7$ calcd: C, 68.24; H, 5.25; obsd: C, 68.46; H, 5.48. Compound **16**: yield: 42%, oil, ^{1}H NMR ($CDCl_3$): 1.25 (t, 3H, C*H*$_3$, 6.5 Hz), 3.82 (s, 6H, 3′ and 5′-OC*H*$_3$), 4.15 (q, br s, 2H, O*CH*$_2$·CH_3, J = 6.5 Hz), 4.74 (br s, 2H, O*CH*$_2$·CH═), 5.80 (d, 1H, ═*CH*CO, J = 15.7 Hz), 6.92 (d, 1H, CH_2·*CH*═, J = 15.7 Hz), 7.14–7.94 (m, 8H, all aromatic protons), FAB mass: 459 $[M+Na]^+$, elemental analysis for $C_{25}H_{24}O_7$, calcd: C, 68.80; H, 5.54; obsd: C, 68.62; H, 5.37.