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Synthesis of 1-(3′,4′,5′-trimethoxy) phenyl naphtho[2,1*b*]furan as a novel anticancer agent☆

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Abstract—3′,4′,5′-Trimethoxy benzoyl-naphthalene 2-*O*-acetic acid (**5**) underwent base catalysed intramolecular condensation to yield exclusively 1-(3′,4′,5′-trimethoxy) phenyl naphtho[2,1-*b*]furan **8**. The cyclised product **8** has been characterised by spectroscopy. The product **8** showed significant anticancer activity against human cancer cell lines COLO320DM (colon), CaCO2 (colon) and WRL68 (liver) at 0.7, 0.65 and 0.50 μg/ml concentrations, respectively, in the in vitro MTT assay.

Furan moieties are common sub-structures in numerous natural products.[2] Naphthofuran derivatives have been isolated from various natural sources like *Fusarium oxysporum*,[3] *Gossypium barbadense*,[4] etc., and are well known for various types of biological activities like antitumour,[5] antifertility,[6] mutagenic,[7] growth inhibitory[8] and oestrogenic.[9] Thus, several approaches have also been developed for their synthesis.[10a–e]

In continuation of our previous work[11] on modification of gallic acid, while preparing the amides of compound **5** as protease inhibitors, it unexpectedly ended as compound **8**. While preparing the amides onto compound **5**, that is, 3′,4′,5′-trimethoxy benzoyl-naphthalene 2-*O*-acetic acid, an intramolecular condensation took place along with elimination of CO_2 and H_2O to yield a cyclised product 1-(3′,4′,5′-trimethoxy) phenyl naphtho[2,1-*b*]furan **8**, which is characterised by spectroscopy. It showed significant anticancer activity against various human cancer cell lines.

Naphthofuran ring was synthesized under basic conditions starting with 3,4,5-trimethoxy benzoic acid. The synthetic scheme is given in Figure 1. The synthesis of compound **5** from gallic acid has already been discussed.[11] Compound **5** along with *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC-HCl), 1-hydroxy benzotriazole (HOBt), triethyl amine and a primary amine under refluxing conditions in dichloromethane yielded exclusively **8** after an intramolecular condensation.[14] The role of primary amine is not clear, but was found to be essential for this condensation. Also, it will be worth mentioning that irrespective of addition of any type of primary amine (aniline, 3,4,5-trimethoxy aniline, *n*-butyl amine, etc.), product **8** was obtained in excellent yields (89%). The cyclisation reaction was further repeated using two more substrates (**6** and **7**) having a similar naphthophenone moiety along with 2-*O*-acetic acid chain to get the desired phenyl naphtho[2,1-*b*]furans (**9** and **10**) in good yields (73–91%). The 2-hydroxy naphthophenone moieties of **6** and **7** were synthesized by stirring the respective acid chlorides (*p*-anisoyl chloride and benzoyl chloride) with 2-methoxy naphthalene in dry dichloromethane at room temperature using anhydrous aluminium chloride.[10e] Rest of the steps were as such of compound **5**.

The structure proposed for compound **8** has been confirmed by spectroscopic means[12] using IR, NMR experiments like ^{1}H NMR, ^{13}C NMR, Dept 135 and 2D (^{1}H–^{1}H and ^{1}H–^{13}C) correlation experiments and finally by electrospray mass spectrometry. The proton spectra taken on 300 MHz FT-NMR in $CDCl_3$ showed two signals at δ 3.79 and 3.89 ppm integrating each for six and three protons which corresponds to a total of three methoxy groups attached to an aromatic ring. A distinct singlet, integrating for one proton resonating

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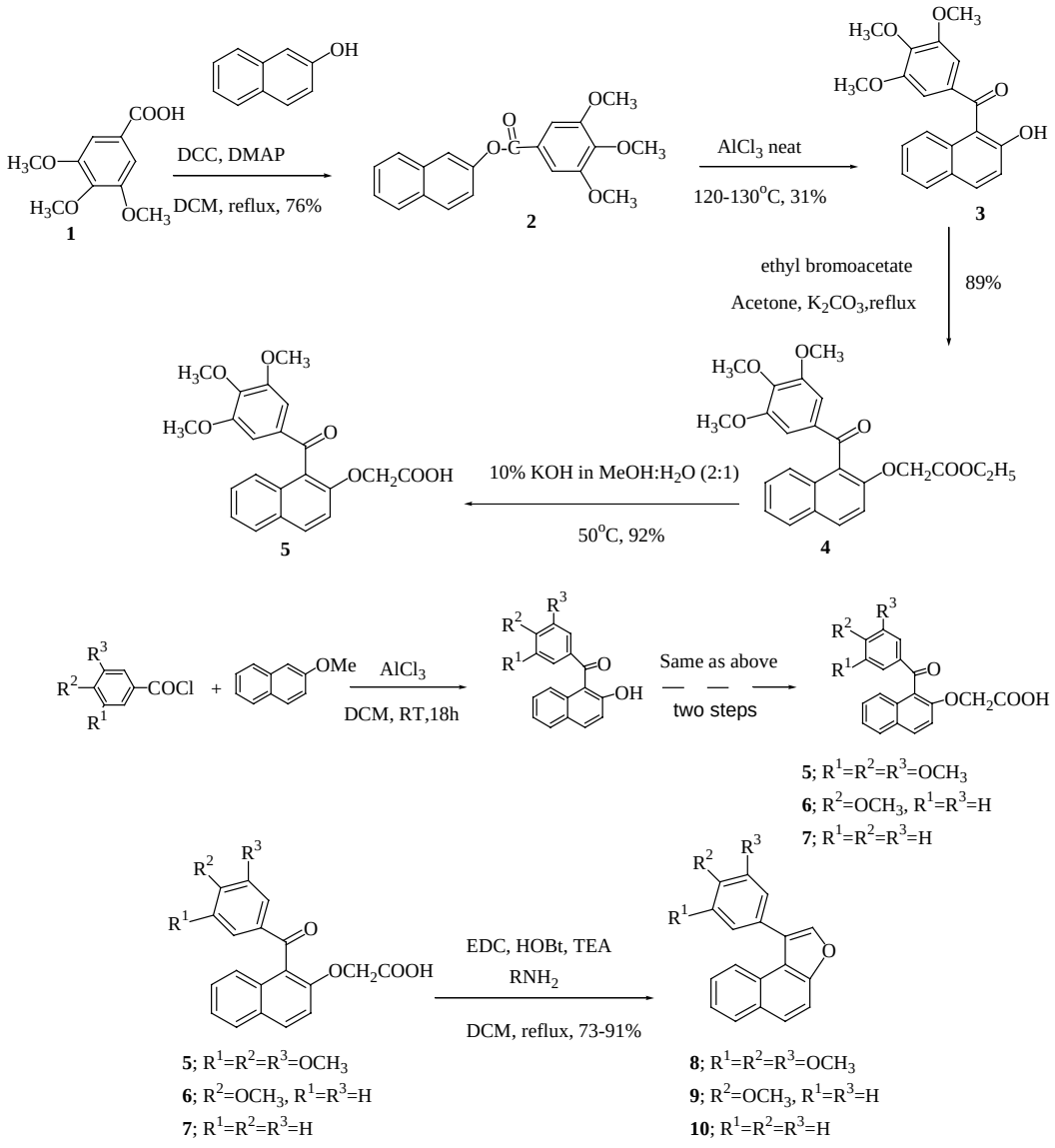

Figure 1. Schematic presentation of synthesis of naphthofurans.

at δ 7.625 ppm, apart from five naphthalenic proton multiplets in this aromatic region, indicates it to be attached on the furan moiety. The ^{13}C NMR spectra showed the presence of total 21 carbons in **8**. ^{13}C NMR coupled with DEPT 135 experiments clearly indicated the presence of 3 methyls (oxygenated), 9 methines (one oxygenated) and 9 quaternary (4 oxygenated) carbons. Absence of both the two carbonyl and one CH_2 carbon resonances in product **8** indicates the possibility of cyclisation through intramolecular condensation along with the elimination of H_2O and CO_2 groups. Further, the special presence of a downfield resonance at δ 141.91 ppm indicates it to be CH of furan moiety and thus confirms the above-said possible cyclisation. Rest of all other carbon resonances are well in agreement with that of the parent and thus, confirm the structure proposed to be that of compound **8**. Further, C–H (^{13}C at δ 141.91 ppm and ^{1}H at δ 7.625 ppm) one bond interaction in HMQC has clearly showed a heteronuclear cross-correlation for CH pair of the furan moiety. Rest of the correlations are well in agreement with that of the parent has supported further the structure proposed. Final authentication for the proposed structure has come from the Mass analysis where the compound showed molecular ion adduct peaks at *m/z* 335.5 $[M+H]^+$, 357.2 $[M+Na]^+$ and 373.1 $[M+K]^+$. IR spectrum showed the absence of carbonyl and hydroxyl groups in **8**. All the above data indicated a possibility of cyclisation within the compound **5**.

The cyclisation might have taken place due to a condensation within the molecule, starting with the abstraction of acidic proton from methylene group of **5** by triethyl amine and subsequent attack of the carbanion at ketonic carbon (naphthophenone) to form a five-membered cyclic system. After this cyclisation, dehydration along with decarboxylation led to a naphthofuran ring **8**. The reaction failed when the free carboxylic acid of **5** was in the ester form (**4**). The possible mechanistic pathway is depicted in Figure 2.

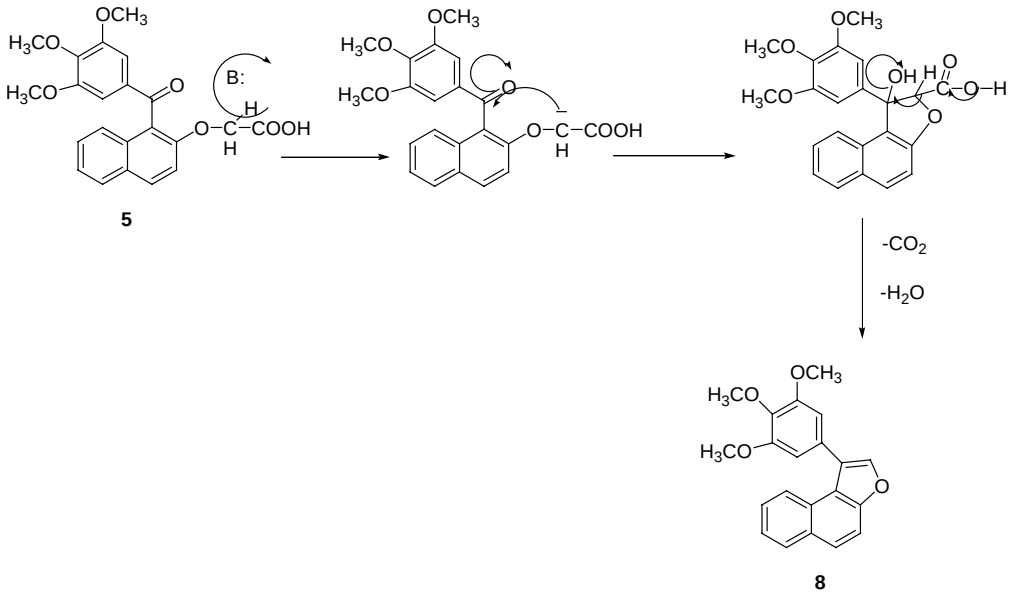

Figure 2. Possible mechanistic pathway of formation of aryl naphthofurans.

Table 1. Cytotoxicity of phenyl naphtho[2,1*b*]furans against various human cancer cell lines by MTT assay

Compound	Cancer cell lines											
	PA1		KB403		WRL68		COLO320DM		CaCO2		MCF7	
	IC_{50} (μg/ml)	IC_{90} (μg/ml)	IC_{50} (μg/ml)	IC_{90} (μg/ml)	IC_{50} (μg/ml)	IC_{90} (μg/ml)	IC_{50} (μg/ml)	IC_{90} (μg/ml)	IC_{50} (μg/ml)	IC_{90} (μg/ml)	IC_{50} (μg/ml)	IC_{90} (μg/ml)
Taxol	0.006	0.9	0.001	0.047	0.0035	2.5	0.0045	0.01	0.007	0.065	0.005	0.85
8	3.5	4.5	8.0	10	0.7	5.0	0.65	4.0	0.50	—	—	—
9	4.0	8.0	—	—	6.0	—	5.0	—	0.95	7.5	—	—
10	—	—	9.0	—	—	—	—	—	—	—	—	—

(—) means inactive.

The anticancer activity of compounds **8**, **9** and **10** using MTT assay[15] is given in Table 1. Compounds were evaluated for in vitro cytotoxic activity against six human cancer cell lines PA1 (ovary cancer cells), KB403 (oral and mouth cancer cells), WRL68 (liver cancer cells), COLO 320DM (colon cancer cells), CaCO2 (colon cancer cells) and MCF7 (hormone dependent breast cancer cells). Taxol (paclitaxel) was used as reference compound.

Compound **8** showed significant cytotoxicity against both colon cancer cell lines, that is, CaCO2 and COLO320DM and WRL68 liver cancer cell lines having IC_{50} values 0.5, 0.65 and 0.7 μg/ml, respectively. Compound **9** also showed good cytotoxic activity against CaCO2 cell lines having an IC_{50} value of 0.95 μg/ml. While compound **10** did not exhibit significant activity against any of the cancer cell lines.

Different benzoyl naphthalene 2-*O*-acetic acid derivatives undergo an intramolecular condensation under EDC, HOBt, TEA and *p*-amine in DCM system to yield the corresponding cyclised product phenyl naphtho[2,1*b*]furans (**8**, **9** and **10**). Among these naphthofurans **8** and **9** showed significant in vitro anticancer activity against various human cancer cell lines. This new methodology will be useful for the synthesis of aryl naphtho[2,1*b*]furans. This study suggests that gallic acid can be modified to produce potent anticancer agents.

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12. Selected physical data.
Compound **8**: yield = 89%; mp = oil, IR = 1582, 1462, 1218, 1127, 770, 670. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.79 (s, 6H, 3′ and 5′ OCH_3), 3.89 (s, 3H, 4′-OCH_3), 6.72 (s, 2H, 2′ and 6′ CH of phenyl ring), 7.34–7.36 (distorted t, 2H, J = 8.19 Hz), 7.57–7.59 (d, 1H, J = 5.79 Hz), 7.625 (s, 1H, CH of furan ring), 7.67–7.70 (d, 1H, J = 9.0 Hz), 7.85–7.87(d, 1H, J = 7.41 Hz), 7.9–8.02 (d, 1H, J = 7.98 Hz); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 153.83, 153.83, 153.55, 141.59, 131.30, 129.32, 129.06, 128.82, 126.39, 125.33, 124.87, 124.79, 123.88, 121.11, 120.62, 113.41, 109.72, 107.81, 61.37, 56.70, 56.70. Electrospray Mass (CH_3CN); *m/z* 335.5 $[M+H]^+$ (86.67%), 357.2 $[M+Na]^+$ (66%), 373.1 $[M+K]^+$ (48.8%), Product ions; 320.1 $[M-14]^+$, 276, 141.1. Elemental analysis calcd for $C_{21}H_{18}O_4$: C, 75.45; H, 5.39. Found: C, 75.86; H, 5.68.
Compound **9**: yield = 91 %; mp = oil, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.83 (s, 3H, OCH_3), 6.96 (d, 2H, 2′ and 6′ CH, J = 8.68 Hz), 7.25–7.37 (m, 2H), 7.42–7.45 (d, 2H, 3′ and 5′ CH, J = 8.65 Hz), 7.58 (d, 1H, J = 5.16 Hz), 7.622 (s, 1H, CH of furan ring), 7.67 (d, 1H, J = 8.97 Hz), 7.86 (d, 1H, J = 7.74 Hz), 7.92 (d, 1H, J = 8.19 Hz); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 159.94, 153.54, 141.94, 131.39, 131.39, 131.26, 129.25, 128.88, 126.29, 126.19, 125.68, 124.66, 124.45, 123.74, 121.42, 114.52, 114.52, 112.99, 55.74. EI-Mass (GC–MS, MeOH); 274 $[M]^+$, Product ions; 259, 202. Elemental analysis calcd for $C_{19}H_{14}O_2$: C, 83.21; H, 5.11. Found: C, 83.44; H, 4.96
Compound **10**: yield = 73%; mp = oil, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.23–7.25 (d, 1H, J = 7.7 Hz), 7.28–7.31 (d,1H, J = 8.25 Hz), 7.33 (d, 2H, J = 7.68 Hz), 7.37–7.38 (m, 1H), 7.44–7.47 (dd, 2H, J = 9.22 and 1.6 Hz), 7.52–7.59 (t, 2H, J = 10.1 Hz), 7.62 (s, 1H, CH of furan ring), 7.76–7.79 (d, 1H, J = 7.9 Hz), 7.86–7.89 (d, 1H, J = 8.2 Hz); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 153.24, 141.67, 133.20, 130.92, 129.89, 129.19, 128.91, 128.57, 128.57, 128.43, 127.85, 125.95, 125.95, 124.52, 124.33, 123.40, 120.80, 112.80. EI-Mass (GC–MS, MeOH);244 $[M]^+$, Product ions; 215, 106. Elemental analysis calcd for $C_{18}H_{12}O$: C, 88.52; H, 4.92. Found: C, 88.76; H, 4.74.
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14. General experimental procedure for the synthesis of aryl naphtho[2,1*b*] furans: Synthesis of 1-(3′,4′,5′-trimethoxy) phenyl naphtho[2,1*b*]furan (**8**). In a 25 ml round-bottomed flask 3′,4′,5′-trimethoxy benzoyl naphthalene 2-*O*-acetic acid (**5**, 100 mg, 0.25 mmol) was taken in dry dichloromethane (10 ml). To this 1-hydroxy benzotriazole (80 mg, 0.63 mmol), EDC-HCl (120 mg, 0.63 mmol) and triethyl amine (0.5 ml) were added. The reaction mixture was stirred at room temperature for 20 min. Now to this mixture 3,4,5-trimethoxy aniline (60 mg, 0.32 mmol) was added and the reaction mixture was refluxed for 2 h. After completion of the reaction, 20 ml water was added to this and stirred for 10 min. It was extracted with dichloromethane (30 ml × 3) and washed with water. The organic layer was dried over anhydrous sodium sulfate and distilled off to get a residue. The residue was passed through a small column of silica gel (60–120 mesh) and eluted with hexane–ethyl acetate. The compound **8** was obtained in 8–10% ethyl acetate–hexane as an oil (89%).
15. In vitro anticancer activity of phenyl naphtho[2,1*b*]furans using MTT assay. Cytotoxicity testing in vitro was done by the method of Woerdenbag et al.[13] 2×10^3 cells/well were incubated in the 5% CO_2 incubator for 24 h to enable them to adhere properly to the 96-well polystyrene microplate (Grenier, Germany). Test compound dissolved in 100% DMSO (Merck, Germany) in at least five doses was added and left for 6 h after which the compound plus media was replaced with fresh media and the cells were incubated for another 48 h in the CO_2 incubator at 37 °C. The concentration of DMSO used in our experiments never exceeded 1.25%, which was found to be non-toxic to cells. Then, 10 μl MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma M 2128] was added, and plates were incubated at 37 °C for 4 h. One hundred microlitres of dimethyl sulfoxide (DMSO, Merck, Germany) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at room temperature to ensure that all crystals were dissolved, the plates were read on a SpectraMax 190 Microplate Elisa reader (Molecular Devices Inc. USA) at 570 nm. Plates were normally read within 1 h of adding the DMSO. The experiment was done in triplicate and the inhibitory concentration (IC) values were calculated as follows:

$$\%\text{inhibition} = [1 - \text{OD}(570\text{ nm}) \text{ of sample well}/\text{OD}(570\text{ nm}) \text{ of control well}] \times 100.$$

IC_{90} is the concentration μg/mL required for 90% inhibition of cell growth as compared to that of untreated control.