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Novel C-9, 9'-O-acyl Esters of (-)-Carinol as Free-radical Scavengers and Xanthine Oxidase Enzyme Inhibitors: Synthesis and Biological Evaluation

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Abstract: New compounds with hydrophyllic esters of (-)-carinol were synthesized and evaluated as xanthine oxidase enzyme inhibitors and antioxidants. Aliphatic esterfication of C-9,9'-OH groups of (-)-carinol resulted in lowering antioxidant and xanthine oxidase inhibitory activities. However certain aromatic acyl esters considerably improved the xathine oxidase inhibition. Aromatic esterification with electron withdrawing substitutions would preferred for improvement in XOD inhibition while retaining radical scavenging activity, electron withdrawing substitution led to the loss of free radical scavenging property and neutral substituents decrease the enzyme inhibitory potential.

Keywords: (-)-Carinol, Acyl derivatives, Xanthine oxidase inhibition, Free radical scavenging activity.

INTRODUCTION

Hyperuricemia is associated with number of pathological conditions such as gout [1] lowering of uric acid level in blood could be achieved by xanthine oxidase inhibitors and inhibitors of renal uratereabsorpotion. Free radical imbalance leads to stress [2] that is being suggested as the root cause of many diseases like atherosclerosis, stroke, diabetes and cancer. XOD is a widely distributed enzyme, especially in the microvascular endothelium. It converts hypoxanthine to xanthine and also xanthine to uric acid, with concomitant production of superoxide anion. This endothelial derived enzyme has received considerable attention as a primary endogenous source of free radicals [3,4] and involves in detoxification, formation of peroxynitirites [5] leading to vascular constriction. Recent studies in CHF (chronic heart failure) patients and models of experimental heart failure showed that xanthine oxidase inhibition increased contractile capacity due to calcium (Ca^{2+}) sensitizing mechanism and improved mitochondrial efficiency by reducing myocardial oxygen consumption. The conclusion might be that targeted XO inhibition as a novel treatment approach might be suitable only in those patients where increased uric acid concentrations are an indicator of up-regulated XO activity. These compelling evidences of increased levels of XOD in various pathological conditions and damages caused by free radicals generated in enzymatic process, warrants search for inhibitors of enzyme that can mitigate over free radicals generation and free radical scavengers that may prevent the damages caused by free radicals generated during enzymatic process. These enzyme inhibitors and free radical scavengers may become important therapeutic agents in mitigating various pathological conditions in different disease process. Free radical generation that exceeds the capacity of antioxidant defenses results in oxidative stress, which possibly elicits irreversible degenerative responses, including apoptosis or necrosis in living cells [6].

Natural products originating from different plants have been observed to possess multiple therapeutic activities [7]. Among them flavonoids have drawn considerable attention in recent past due to their antioxidant and multiple enzyme inhibitory activities. Flavonoids have been studied extensively for their free radical scavenging and XOD inhibitory activities [8,9]. However, least is reported about free radical scavenging and XOD inhibitory activities of other class of compounds, like lignans. In the course of our biological activity-screening program from Indian medicinal plants for identifying free-radical scavengers [10,11] and XOD inhibitors, [12] we observed several lignans possessing mild to moderate XOD inhibitory activities [13]. Recent reports also proved that C-9,9'-O-acyl esters of lignans possesses strong cytotoxic activity against human lung carcinoma and breast carcinoma cell lines [14].

In continuation, the present study reports the synthesis, biological evaluation, and structure activity relationship for novel C-9,9'-O-acyl esters of (-)-carinol and also explores the possibilities of common structural requirement that may possess better XOD inhibitory activity coupled with free radical scavenging property.

MATERIALS AND METHODS

(-)- Nortrachelogenin was isolated from a wellknown Indian medicinal plant *Cedrus deodara* in substantial yields

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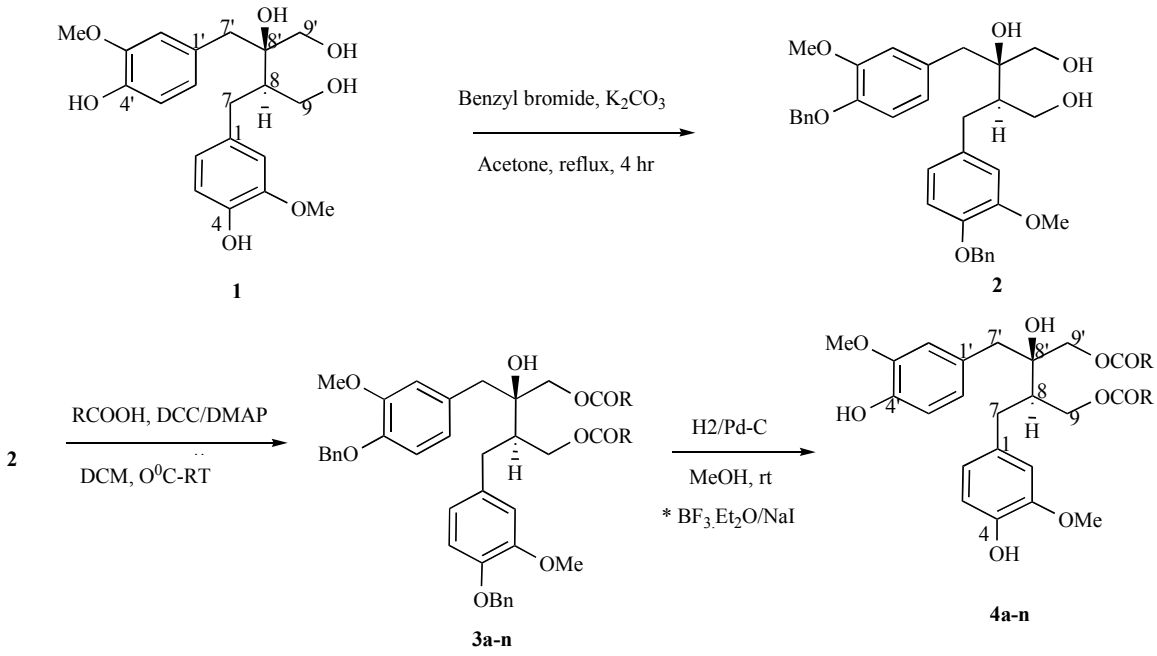

1 **2** **3a-n** **4a-n**

3a. R = -CH_3
3b. R = -$(CH_2)_2CH_3$
3c. R = -$(CH_2)_4CH_3$
3d. R = -$(CH_2)_6CH_3$
3e. R = -$(CH_2)_{10}CH_3$
3f. R = -$(CH_2)_{12}CH_3$
3g. R = -$(CH_2)_{14}CH_3$
3h. R = -Ph
***3i.** R = -(CH=CH)Ph
3j. R = -(*p*-OMe)Ph
3k. R = -(*p*-NO_2)Ph
3l. R = -(*p*-F)Ph
3n. R = -(3,4,5-OMe)Ph
3m. R = -(*p*-OH,*m*-OMe)Ph

4a. R = -CH_3
4b. R = -$(CH_2)_2CH_3$
4c. R = -$(CH_2)_4CH_3$
4d. R = -$(CH_2)_6CH_3$
4e. R = -$(CH_2)_{10}CH_3$
4f. R = -$(CH_2)_{12}CH_3$
4g. R = -$(CH_2)_{14}CH_3$
4h. R = -Ph
4i. R = -(CH=CH)Ph
4j. R = -(*p*-OMe)Ph
4k. R = -(*p*-NO_2)Ph
4l. R = -(*p*-F)Ph
4m. R = -(*p*-OH,*m*-OMe)Ph
4n. R = -(3,4,5-OMe)Ph

Scheme 1. Synthesis of 9, 9'-O-acyl esters of (-)-carinol.

that displayed potent free radical (DPPH) scavenging activity [14] and mild XOD inhibitory activity. Interestingly, when
(-)-Nortrachelogenin was converted into (-)-carinol, [15] the XOD inhibitory activity level increased substantially. In view of available literature on lignan derivatives, our strategy for the synthesis of (-)-carinol derivatives relies upon retaining the phenolic hydroxyl groups and modification of terminal alcohol groups (C-9, 9'). This was achieved by the protection of two phenolic hydroxyl groups as their benzyl ethers (**2**) and subsequent condensation with corresponding acid in presence of dicylohexyldicarbodiimide (DCC) and dimethylaminopyridine (DMAP) in anhydrous methylene chloride to obtain the key intermediate (**3a-n**) further debenzylation using palladium on carbon under hydrogen atmosphere to obtain the target compounds in excellent yields (Scheme **1**). All the synthesized compounds were purified and characterized using classical spectroscopic methods such as IR, Mass and NMR.

RESULTS AND DISCUSSION

In view of our recent observations and the available literature suggested that the peroxyl radical should be the major target for radical scavenging antioxidants. Therefore electron donating substituent's on the aromatic ring of the phenolic pharmacore increases the reactivity towards peroxyl radicals and also increases the –OH bond dissociation energies, whereas electron withdrawing substituent's tends to decrease it. Therefore, electron donating groups are important substituent for a compound to possess antioxidant activity.

Diphenylpicrylhydrazyl (DPPH) is a nitrogen centered free-radical that reacts similar to peroxyl radicals and its reaction rates correlates directly with antioxidant activity [16].Therefore, we chose DPPH as a test model in our study to evaluate free-radical scavenging activity of the compound that representperoxyl radical scavenging in-vivo. Influenced with these insights, we prepared seven aliphatic esters with different electron withdrawing and electron donating substituents and seven aromatic 9-9'-O-acyl ester derivatives of (-)-carinol, respectively. The primary results for their free radical DPPH scavenging and XOD inhibitory activity are presented in (Table **1**). It is interesting to note from Table **1** that both aliphatic and the aromatic esterification at C-9,9'-O-acyl positions decreases DPPH scavenging property of the carinol derivatives. Furthermore, substitution of electron withdrawing groups (**4k, 4l**) and neutral group (**4h**) led to the loss of DPPH scavenging property. These observations clearly demonstrate that apart from the presence of vanilloyl-OH in divanilyllignans, which contribute to the free-radical scavenging activity in lignans, presence of free hydrogen donating groups (OH) at 9, 9'-O-acyl positions increase their

Table 1. Antioxidant (DPPH) Activity and Xanthine Oxidase Enzyme Inhibition of (-)-Carinol (1) and its 9, 9′-O-acyl Esters (4a-n)[a]

Compound	%DPPH Scavenging At 25 µg/mL	% of Xanthine Oxidase Inhibition at 100 µg/mL
4a	45.67±1.2	12.92±0.5
4b	54.97±1.3	13.18±0.4
4c	51.02±2.3	31.16±0.5
4d	55.42±0.6	17.12±0.5
4e	66.56±0.6	12.73±0.2
4f	51.97±0.7	10.63±0.5
4g	41.82±0.2	25.25±1.1
4h	NA	18.74±0.0
4i	53.34±1.1	19.12±0.1
4j	47.95±0.4	40.31±0.2
4k	NA	52.19±0.7
4l	NA	39.96±0.0
4m	40.00±2.5	54.30±3.5
4n	40.83±0.3	32.37±1.4
Carinol (1)	68.21±0.0	36.56±0.4

[a]Values represent mean and SD of triplicate samples.

Table 2. IC_{50} and SC_{50} Values of Compounds for their Xanthine Oxidase Inhibitory and DPPH Scavenging Activities

Compound	IC_{50} (µg/ml) for XOD	SC_{50} (µg/ml) for DPPH
Carinol	219.4	4.4
4j	145.5	191.7
4k	88.5	NA
4l	157.1	NA
4m	93.9	67.4
4n	135.5	135.5
Ascorbic acid	NA	5.1
Allopurinol	1.7	NA

IC_{50} value is the concentration of compound required to inhibit 50% activity of XOD, SC_{50} value is the concentration of compounds required to scavenging 50% of DPPH radical. These values were obtained from linear regression analysis of the mean values of triplicate samples. NA- Not active. Ascorbic acid was chosen as reference compound for DPPH scavenging activity and allopurinol for xanthine oxidase activity.

free-radical scavenging activity and are better natural substituents than electron donating substituents **(4J, 4m, and 4n)**.

Similarly, aliphatic esterification of (-)-carinol **(4a-4g)** also significantly decreased XOD inhibitory activity **(**Table **1)**. However, aromatic esterification with electron donating **(4j, 4m)** and electron withdrawing substituents **(4k, 4l)** considerably improved the XOD inhibitory activity. Substitution with rather neutral groups **(4h, 4i)** decreased the enzyme inhibitory potential (Table **1**). Though electron-withdrawing substituents (**4k** and **4l**) improved the enzyme inhibitory potential of derivatives, it led to the loss of DPPH scavenging activity. Therefore substituents with hydrogen and or electron donating or both the properties, appear preferable to retain both the activities (**4m, 4n, 4j**).

Despite the improvement in XOD inhibitory activity by electron-withdrawing groups, substitution with hydrogen, electron donating and or both appears preferable, as former leads to the loss of free radical scavenging property. This study shows that presence and number of hydrogen donating groups at 9-9' positions improve DPPH scavenging activity and aromatic esterification preferably with electron donating substituent at these positions improves XOD inhibitory potential of the compounds with retention of free radical scavenging property. We first time demonstrated that (-)-carinol acyl esters are potent inhibitors of xanthine oxidase with together with anti-oxidant properties.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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