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Novel 3-*O*-Acyl Mesquitol Analogues as Free-Radical Scavengers and Enzyme Inhibitors: Synthesis, Biological Evaluation and Structure–Activity Relationship

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Abstract—A new isomer of mesquitol (2,3-*trans*-3′,4′,7,8-tetrahydroxyflavan-3-ol) was isolated from *Dichrostachys cinerea* in excellent yields. It has shown free-radical scavenging property and α-glucosidase inhibitory activities but, it could not display xanthine oxidase inhibitory property. However, it was observed that acylation of 3-OH group significantly enhanced the α-glucosidase inhibition and displayed xanthine oxidase inhibitory potential. The structure activity relationship revealed that the degree of lipophilicity played a major role in improving enzyme inhibitory activities. A positive correlation was observed between enzyme inhibitory potential and acyl chain length (upto C-16) of aliphatic esters.
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Drugs with antioxidant mechanisms are being widely proposed as bases for the development of new approaches for pharmacological regulation of peroxidative-antioxidative homeostatic imbalance which is responsible for development of several disorders.[1] Antioxidant molecules isolated from natural products are being reported to possess multiplicity in their activities either directly related to their antioxidant property or by other mechanisms.[2] Studies on the free-radical scavenging properties of flavonoids have permitted characterization of the major phenolic components of naturally occurring phytochemicals as antioxidants.[3] Flavans, an important class of natural antioxidants, have been reviewed to possess a number of enzyme inhibitory properties related to various disease conditions.[4]

Xanthine oxidase is considered to be the important biological source of free radicals.[3] Also, there is overwhelming acceptance that xanthine oxidase serum levels are significantly increased in various pathological states like hepatitis, inflammation, hypercholesterolemia, atherosclerosis, ischemia-reperfusion, carcinogenesis and aging and that free radicals generated in the enzymatic process are involved in oxidative damage.[5] Thus, it may be possible that the inhibition of this enzymatic pathway for instance by compounds that have simultaneously antiradical and xanthine oxidase inhibitory properties could have therapeutic interest. It has been reported that some flavonoids and structurally related antioxidants inhibit xanthine oxidase.[6]

Similarly, it is known for a long time that the levels of glycosidase are elevated in the sera of many patients with different tumors[7] and inhibitors of glucosidases, particularly α-glycosidase inhibitors possess potential of broad spectrum therapeutic activities like anti viral, anti cancer and anti diabetic.[8] It has also been reported that radical scavenging α-glucosidase inhibitory constituents from medicinal plants possess variety of biological activities like anti HIV, antiviral and anti-inflammatory etc.[9]

In this letter we report the isolation of a new isomer of mesquitol **1** (2,3-*trans*-3′,4′,7,8-tetrahydroxyflavan-3-ol) from traditional Indian medicinal plant *Dichrostachys cinerea*[10,11] in substantial yield[12] along with minor

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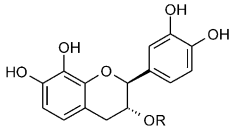
amount of (−)-epicatechin (0.2%). The structure of (−)-mesquitol was established using physical and spectroscopic methods.[13] The 3′,4′-dihydroxyphenyl group at C-2 was in *trans* position to the hydroxyl group at C-3 according to the large coupling constant of the H-2 signal ($J=7.25$ Hz) which was coincident with that of a (+)-mesquitol moiety ($J_{H\text{-}2}=7.25$ Hz).[14] The specific rotation of the compound has shown opposite sign to that of (+)-mesquitol. Hence, the configuration at C-2 and C-3 is assumed as 2*S*, 3*R*.

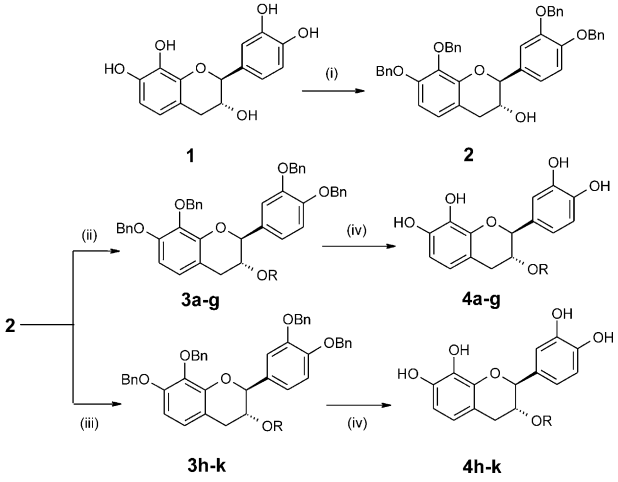
In our screening (−)-mesquitol showed better level of free-radical scavenging and α-glucosidase inhibitory activity than (−)-epicatechin (Table 1). The absence of xanthine oxidase inhibition for (−)-mesquitol and (−)-epicatechin may be due to the presence of free hydroxyl group at C-3 position.[15] It is well documented that radical scavenging activity of antioxidant molecules is primarily due to the pyrogallol/catechol moiety in the molecules. Further the addition of alkyl groups introduces enzyme inhibition properties of the molecule and that inhibition of the enzyme increases with increase in the alkyl chain length.[16] Since, (−)-mesquitol has elicited better free-radical scavenging and α-glucosidase inhibitory activity than (−)-epicatechin and that the presence of free hydroxyl group at C-3 position inhibit it to elicit xanthine oxidase inhibitory activity, we aimed to prepare 3-*O*-aliphatic acyl esters of (−)-mesquitol in order to introduce xanthine oxidase inhibitory property as well as to improve its enzyme inhibitory potential. Simultaneously, we also prepared some 3-*O*-aromatic acyl esters of (−)-mesquitol in order to make comparative analysis (Fig. 1).

The aliphatic esters were prepared (Scheme 1) by condensing the corresponding aliphatic acids with (−)-3′,4′,7,8-tetra-*O*-benzyl mesquitol **2** in the presence of dicyclohexyl carbodiimide in anhydrous methylene chloride[17] fallowed by debenzylation using palladium on carbon under hydrogen atmosphere to yield the target compounds (**4a–g**) in 85%. The aromatic esters were obtained (Scheme 1) by treating the (−)-3′,4′,7,8-tetra-*O*-benzyl mesquitol **2** in methylene chloride with the corresponding aromatic acid chloride in the presence of triethylamine followed by debenzylation to



1 R = H,
4a. R = -$COCH_3$
4b. R = -CO $(CH_2)_2CH_3$
4c. R = -CO $(CH_2)_4CH_3$
4d. R = -CO $(CH_2)_8CH_3$
4e. R = -CO $(CH_2)_{12}CH_3$
4f. R = -CO $(CH_2)_{14}CH_3$
4g. R = -CO $(CH_2)_{16}CH_3$
4h. R = -COPh
4i. R = -CO(*o*-Cl)Ph
4j. R = -CO(*p*-OCH_3)Ph
4k. R = -CO(*p*-F)Ph

Figure 1.



Scheme 1. Reagents and conditions: (i) $PhCH_2Br$, K_2CO_3, acetone, reflux; (ii) corresponding aliphatic acid, DCC, DMAP, CH_2Cl_2; (iii) corresponding aromatic acid chloride, Et_3N, CH_2Cl_2; (iv) H_2, 10% Pd-C, MeOH.

Table 1. Free-radical scavenging activity (DPPH, ABTS), α-glucosidase and xanthine oxidase inhibition of (−)-mesquitol and its 3-*O*-acyl esters

Compd	Free-radical scavenging activity, SC_{50} (μM)		α-glucosidase inhibition	xanthineoxidase inhibition
	DPPH	ABTS	IC_{50} (μM)	IC_{50} (μM)
1	6.72	14.59	82.32	NA
4a	7.15	13.04	46.31	150
4b	8.89	14.09	46.18	139
4c	6.68	14.41	49.34	129
4d	6.07	17.57	13.46	112
4e	7.51	11.29	12.56	102
4f	5.17	10.05	9.56	94.7
4g	9.43	17.75	54.45	155.75
4h	11.53	—	77.76	NA
4i	7.97	—	43.26	NA
4j	6.72	—	67.26	NA
4k	8.99	—	32.82	NA
(−)-Epicatechin	13.47	—	177.56	NA
Probucol	21.67	—	—	—
Trolox	15.94	1.82	—	—
1-Deoxynoji rimycin	—	—	50.00	—
Allopurinol	—	—	—	34.91

NA, (Compounds were considered not active at concentration 50 μg/mL giving activity less than 15%), IC_{50} or SC_{50} values were determined by linear regression analysis using at least five different concentrations in triplicate and represent mean of the experiment, SDM were within 10% in any case, — Not tested.

yield the compounds (**4h–k**) in 80%. All the esters were purified and characterized using spectroscopic methods[18] before subjecting to biological evaluation.

(−)-Mesquitol and its 3-*O*-acyl esters were screened for free-radical scavenging (DPPH, ABTS) activities.[19,20] It is evident from the present study (Table 1) that the antioxidant activity is due the phenolic hydroxyl groups present on the ring A and B. Although, (−)-mesquitol and its acyl esters regardless of their acyl substitution, showed potent scavenging activity on the DPPH and ABTS radical, compounds showed more potential radical scavenging activity on lipophilic free radical, DPPH than the hydrophilic free radical, ABTS (Table 1).

It is well documented that the presence of free hydroxyl group at C-3 position in flavonoids reduces the xanthine oxidase enzyme inhibition to a greater extent.[15] All the aliphatic and aromatic 3-*O*-acyl esters were tested[21] for xanthine oxidase inhibitory activity. The aliphatic 3-*O*-acyl esters showed better inhibition pattern. The trend observed in the experiments reported in Table 1 could be explained on the basis of degree of lipophilicity of the compounds. Consequently, by elongating the alkyl chain in the acyl group, a better affinity was favored for the space around the active site of the enzyme. When the IC_{50} values of selected aliphatic esters were plotted against the length of the acyl chain a linear correlation was observed (Fig. 2). The influence of acyl substitution on the xanthine oxidase inhibition is well noticed as indicated by lack of activity for (−)-mesquitol. Surprisingly, none of the aromatic analogues could show any inhibition.

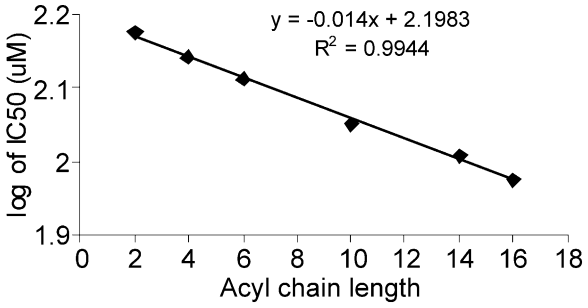


Figure 2. Xanthine oxidase inhibitory potential of 3-*O*-acyl esters of (−)-mesquitol (**4a–f**). The inhibitory potential of esters increased with the length of acyl chain as indicated by good linear correlation ($r^2 = 0.9951$).

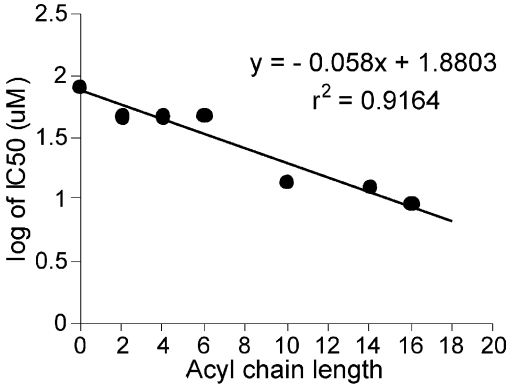


Fig 3. α-Glucosidase inhibitory potential of (−)-mesquitol **1** and its esters (**4a–f**). The 3-*O*-acyl esters are more potent than the parent compound. In particular the inhibitory potential of 3-*O*-acyl esters increased with the length of the acyl chain as indicated by the good linearity ($r^2 = 0.9164$).

(−)-Mesquitol and its 3-*O*-acyl derivatives were assayed[22] for α-glucosidase enzyme inhibition wherein the IC_{50} values are found to be in the range 82.3 to 9.5 μM (Table 1). It is plausible that the inhibitory effect of (−)-mesquitol may be related to the phenolic hydroxyls located on its flavan framework. The introduction of an acyl group on the C-3 position has enhanced the inhibitory potential to a greater extent. However, by increasing the length of acyl group of aliphatic esters (**4a–g**) a better affinity is favored. When the IC_{50} values were plotted against the length of the acyl chain a linear correlation was observed up to a length of C-16 (Fig. 3). All the aromatic esters of (−)-mesquitol (**4h–k**) have shown better inhibitory potential than the parent compound.

In summary, a novel isomer of mesquitol is isolated in excellent yield from *Dichrostachys cinerea*. The better and improved α-glucosidase inhibitory potential, incorporation and increase in xanthine oxidase inhibitory properties along with increase in lipophilicity of (−)-mesquitol esters (**4a–g**) without affecting their free radical scavenging potential therefore may offer exciting opportunity for their development as therapeutic agents in disorders where increase in free radical generation, xanthine oxidase and α-glucosidase levels play an important role in pathogenesis.

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13. **(−)-Mesquitol 1.** Amorphous powder; mp 252 °C; $[\alpha]_D$ −36.05 (c 1.0, MeOH); ^{1}H NMR [200 MHz; $(CD_3)_2CO$] δ 7.95, 7.93, 7.25 and 7.55 (each s, 4×Ar-OH), 6.88–6.72 (3H, m, H-2′,5′,6′), 6.40 (2H, s, H-5,6), 4.62 (1H, d, J=7.5 Hz, H-2), 4.01 (1H, br s, OH-3), 4.01 (1H, m, H-3), 2.89 (1H, dd, J=5 and 15 Hz, H-4_{eq}), 2.71 (1H, dd, J=8 and 15.0 Hz, H-4_{ax}); ^{13}C NMR [50 MHz; $(CD_3)_2CO$] δ 32.08 (C-4), 66.49 (C-3), 81.08 (C-2), 108.07 (C-6), 112.07 (C-4a), 114.39 (C-5), 115.20 (C-5′), 118.20 (C-6′), 118.68 (C-2′), 130.89 (C-1′), 132.85 (C-8), 144.17 (C-7), 144.86 (C-8a), 144.94 (C-3′, 4′); HRFABMS: $[M+H]^+$ 291.0860 calcd for $C_{15}H_{14}O_6$, 291.0868.
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