# α-Glucosidase Inhibitory Constituents from *Cuscuta reflexa*

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**Two new compounds, 7′-(3′,4′-dihydroxyphenyl)-*N*-[(4-methoxyphenyl)ethyl]propenamide (4), and 7′-(4′-hydroxy,3′-methoxyphenyl)-*N*-[(4-butylphenyl)ethyl]propenamide (5) have been isolated from *Cuscuta reflexa* along with five known compounds, 6,7-dimethoxy-2*H*-1-benzopyran-2-one (1), 3-(3,4-dihydroxyphenyl)-2-propen-1-ethanoate (2), 6,7,8-trimethoxy-2*H*-1-benzopyran-2-one (3), 3-(4-*O*-β-D-glucopyranoside-3,5-dimethoxyphenyl)-2-propen-1-ol (6), 2-(3-hydroxy-4-methoxyphenyl)-3,5-dihydroxy-7-*O*-β-D-glucopyranoside-4*H*-1-benzopyrane-4-one (7), reported for the first time from this species. Structures of these compounds were determined by spectral analysis. These compounds showed strong inhibitory activity against α-glucosidase.**

**Key words** *Cuscuta reflexa*; Convolvulaceae; enzyme inhibitor

The genus *Cuscuta* belonging to the family Convolvulaceae, comprises three species. All of these are leafless, twining parasites with slender yellowish stems,[1] distributed in tropical and temperate regions. *Cuscuta reflexa* ROXB. is a leafless parasitic climber which is commonly found in Karachi and other parts of Pakistan. This plant is depurative and purgative and is used in retention of wind and induration of liver while externally used against itches and skin diseases.[2] Previously, long chain esters of the oleane series and sterol glycosides have been reported from this plant.[3] The methanolic extract and its subsequent ethyl acetate fraction showed significant inhibition against α-glucosidase, while against other enzymes (thrombin, β-glucuronidase) gave negative results. α-Glucosidase is a membrane bound enzyme at the epithelium of the small intestine. Inhibition of this enzyme prolongs the absorption time of glucose in the blood after a meal.[4] Thus, post prandial hyperglycaemia can be decreased and in this way non-insulin dependent diabetes (NIDDM) can be managed. This prompted us to carry out further phytochemical studies on this plant. In this paper we describe the isolation and structural elucidation of two new compounds namely, 7′-(3′,4′-dihydroxyphenyl)-*N*-[(4-methoxyphenyl)ethyl]propenamide (**4**) and 7′-(4′-hydroxy,3′-methoxyphenyl)-*N*-[(4-butylphenyl)ethyl]propenamide (**5**) together with five known compounds, 6,7-dimethoxy-2*H*-1-benzopyran-2-one (**1**),[5] 3-(3,4-dihydroxyphenyl)-2-propen-1-ethanoate (**2**),[6] 6,7,8-trimethoxy-2*H*-1-benzopyran-2-one (**3**),[7] 3-(4-*O*-β-D-glucopyranoside-3,5-dimethoxyphenyl)-2-propen-1-ol (**6**)[8] and 2-(3-hydroxy-4-methoxyphenyl)-3,5-dihydroxy-7-*O*-β-D-glucopyranoside-4*H*-1-benzopyrane-4-one (**7**),[9] reported for the first time from this species. All of these compounds have been isolated from the hexane insoluble residue of the ethyl acetate fraction obtained from fractionation of the methanolic extract, followed by repeated column chromatography over silica gel, as described in the Experimental.

Compound **4** was assigned the molecular formula $C_{18}H_{19}NO_4$ by high resolution (HR)-MS, which showed a $[M]^+$ peak at *m*/*z* 313.1285 (Calcd 313.1292). The molecular ion peak was confirmed by FAB-MS which showed $[M+H]^+$ and $[M+Na]^+$ peaks at *m*/*z* 314 and 336, respectively. The IR spectrum suggested the presence of amide (3280 $cm^{-1}$), α,β-unsaturated carbonyl group (1650 $cm^{-1}$) and hydroxyl group (3550 $cm^{-1}$).[10] The UV spectrum showed strong absorption maxima at $\lambda_{max}$ 320, 290 and 225 nm, indicating a highly conjugated system.[10] The MS of **4** displayed an ion peak at *m*/*z* 178 which was due to the loss of $C_8H_7O_2$ from the molecular ion. The ion at *m*/*z* 163 was assigned to the fragment $C_9H_7O_3$. The fragment at *m*/*z* 136 corresponded to $C_8H_8O_2$, resulting from the cleavage of the bond between (C-8′) and the carbonyl bond. This fragmentation pattern suggested that the two hydroxyl groups were in ring A.[11] This was also confirmed by acetylation which gave the diacetate **4a**. The $^1$H-NMR spectrum of **4** showed a signal at δ 3.78 due to a methoxyl group and further signals for methylene groups at δ 2.66 (2H, t, *J*=7.1 Hz) and δ 3.39 (2H, t, *J*=7.1 Hz). The two doublets at δ 6.92 and 6.93 (d, 2H, *J*=8.3 Hz) were assigned to H-2/H-6 and H-3/H-5 hydrogens. The presence of a disubstituted cinnamate group was confirmed by ABX-type signals at δ 6.89 (d, *J*=1.7 Hz,) 6.78 (d, *J*=8.1 Hz,), 6.87 (dd, *J*=8.1, 1.7 Hz) together with the olefinic proton signals at δ 7.33 (d, *J*=15.6 Hz) and 6.13 (d, *J*=15.5 Hz) attributable to H-2′, 5′, 6′, 7′ and 8′, respectively.[12] The broad band and distortionless enhancement by polarization transfer (DEPT) $^{13}$C-NMR spectra showed the presence of one methyl, two methylene, nine methine, and six quaternary carbons which provided further evidence for the presence of disubstituted cinnamate and amide functionalities.[5] The position of the methoxyl group was established at (C-4) through a heteronuclear multiple bond connectivity (HMBC) experiment in which the methoxyl group (δ 3.78) showed $^3J$ interaction with C-4 (δ 160.1). It was also confirmed by nuclear Overhauser effect spectroscopy (NOESY) experiments which showed a cross peak between the methoxyl at δ 3.78 and H-3 and H-5 at δ 6.93.

Compound **5** was assigned the molecular formula $C_{22}H_{27}NO_3$ by HR-MS. The IR and UV showed close resemblance to those of **4**. Detailed $^1$H-NMR study revealed the same substitution pattern in ring A and B, in addition to signals at δ 0.75 and 1.13 due to an additional alkyl side chain. Acetylation of **5** afforded the monoacetate **5a** confirming the presence of one hydroxyl group. The mass fragmentation

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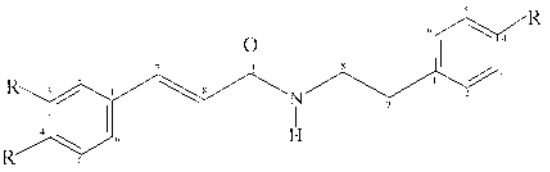

4, R = R = OH R = $OCH_3$
4a, R = R = OAc R = $OCH_3$
5, R = $OCH_3$, R = OH, R = $(CH_2)_3CH_3$
5a, R = $OCH_3$, R = OAc, R = $(CH_2)_3CH_3$

pattern was similar to **4**, showing ions at *m/z* 353 $[M]^+$, 204 $[M^+-C_9H_9O_2]$, 177 $[M^+-C_{12}H_{18}N]$, 176 $[M^+-C_{10}H_9O_3]$, 150 $[M^+-C_{13}H_{18}NO+H]$, 136 $[M^+-C_{14}H_{19}NO]$. The spectrum revealed that **5** has a structure similar to **4** with a major difference in substitution in ring B. In **5**, the methoxyl group in ring B of **4** was replaced by an *n*-butyl group and one of the hydroxyl groups was also methylated. The broad band and DEPT $^{13}$C-NMR spectra showed the presence of two methyl, five methylene, nine methine and six quaternary carbons which supported the above substitution pattern. The positions of the methoxyl and butyl groups were confirmed through an HMBC experiment. The methoxyl at $\delta$ 3.75 showed a $^3J$ correlation with C-3′ ($\delta$ 148.9) while the protons at $\delta$ 1.13 showed a $^2J$ correlation with C-4 ($\delta$ 135.5) and a $^3J$ correlation with C-3 and C-5 at ($\delta$ 129.9). The NOESY spectrum of **5** showed a cross peak between the methoxyl group at $\delta$ 3.75 and H-2′ at $\delta$ 6.92 confirming the presence of a methoxyl group at (C-3′).

All the isolated compounds were subjected to enzyme inhibition studies against $\alpha$-glucosidase type VI[13,14] using 1-deoxynojirimycin as control ($IC_{50}$=0.3 mM). The Compounds **4** and **5** showed strong inhibitory activity ($IC_{50}$=103.58, 45.67 $\mu$M respectively) while compounds **1** and **7** showed moderate activity ($IC_{50}$=0.44, 0.24 mM respectively). Compounds **2**, **3** and **6** were inactive. None of these compounds showed activity against thrombin and $\beta$-glucuronidase.

**Experimental**

Melting points were uncorrected. UV were determined in MeOH on a Pye Unicam SP-800 spectrophotometer and IR spectrum was carried out in $CHCl_3$ on a Shimadzu IR spectrophotometer IR 460. EI-MS and HR-FAB-MS were recorded on a JMS-DZ 500 mass spectrometer. $^1$H-, $^{13}$C-NMR, $^1$H-detected heteronuclear multiple quantum coherence (HMQC) and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for $^1$H- and 75 MHz for $^{13}$C-NMR, respectively. Samples were recorded in $CDCl_3$. Chemical shift values ($\delta$) are relative to tetramethylsilane (TMS) as an internal standard.

**Plant Material** The plant material was collected from the Karachi region from *Melia azadirachta* and Q*uercus brandisiana* trees were identified as *Cuscuta reflexa* Roxb. by Prof. M. Qaiser, Department of Botany, University of Karachi, Pakistan. A voucher specimen is deposited in the Herbarium of the Department of Botany, University of Karachi, Pakistan.

**Extraction and Isolation** The shade dried plant material (30 kg) was extracted thrice at room temperature with MeOH. The methanolic extract was partitioned between ethyl acetate and water. The ethyl acetate fraction was evaporated and divided into hexane soluble and hexane insoluble fractions. The hexane insoluble fraction was evaporated and the residue was chromatographed over silica gel using various mixtures of hexane, chloroform and methanol. The fraction which eluted with $CHCl_3$ gave three major spots and was further purified by chromatography over silica gel using *n*-hexane–ethyl acetate (1 : 1) as eluent to obtain **1** and **2**. The fraction which eluted with $CHCl_3$–MeOH (20 : 1) gave one major spot which was further purified by chromatography over silica gel using *n*-hexane–ethylacetate (1 : 1) as eluent to provide **3**. The fraction which eluted with $CHCl_3$–MeOH (19 : 1) gave two major spots which on further purification over silica gel using hexane–ethyl acetate as eluent provided **4** and **5**. The fraction eluted with $CHCl_3$–MeOH (9 : 1) gave one major spot, which was further purified by preparative TLC in the solvent system $CHCl_3$–MeOH (8 : 2) to give compound **6**. The fraction which eluted with $CHCl_3$–MeOH (7 : 3) gave one major spot which crystallized from MeOH to provide **7**.

Acetylation of **4** and **5**: Compounds **4** (20 mg) and **5** (15 mg) were separately dissolved in pyridine (1 ml) with $Ac_2O$ (3 ml), and the mixture left overnight at room temperature. Ice was added to the reaction mixture and the resulting mixture extracted with EtOAc. The EtOAc layer was evaporated to yield the diacetate **4a** and monoacetate **5a**, respectively.

Compound **4**: Colourless solid. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 320 (4.43), 290 (3.96), 225 (4.12). IR ($CHCl_3$) $cm^{-1}$: 3550, 3280, 1650, 888, 810. $^1$H-NMR ($CDCl_3$+$CD_3OD$, 400 MHz) $\delta$: 7.33 (1H, d, *J*=15.6 Hz, H-7′), 6.93 (2H, d, *J*=8.3 Hz, H-3, 5), 6.92 (2H, d, *J*=8.3 Hz, H-2, 6), 6.89 (1H, d, *J*=1.7 Hz, H-2′), 6.87 (1H, dd, *J*=8.1, 1.7 Hz, H-6′), 6.78 (1H, d, *J*=8.2 Hz, H-5′), 6.13 (1H, d, *J*=15.5 Hz, H-8′), 3.78 (3H, s, OMe), 3.39 (2H, t, *J*=7.08 Hz, H-8), 2.66 (2H, t, *J*=7.1 Hz H-7). $^{13}$C-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 168.4 (C=O), 160.1 (C-4), 145.9 (C-4′), 141.0 ( C-7′), 130.2 (C-6), 130.2 (C-2), 127.8 (C-1′), 127.7 (C-1), 121.4, (C-6′), 118.4 (C-8′), 116.1 (C-5′), 115.8 (C-2′), 114.9 (C-3′), 114.1 (C-3), 114.1 (C-5), 56.2 (OMe), 41.9 (C-8), 35.2 (C-7). MS *m/z*: 313 $[M]^+$ (25), 178 (100), 163 (35), 151 (20), 136 (40), 123 (44). HR-MS *m/z* 313.1295 (Calcd for $C_{18}H_{19}NO_4$, 313.1292).

Compound **4a**: Solid, $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$: 7.35 (1H, d), 6.94 (2H, d), 6.89 (2H, d), 6.87 (1H, d), 6.85 (1H, dd), 6.79 (1H, d), 6.15 (1H, d), 3.80 (3H, s), 3.50 (2H, t), 2.71 (2H, t), 2.23 (6H, s), 2.31 (3H, s). HR-FAB-MS *m/z*: 398.1634 (Calcd for $C_{22}H_{24}NO_6$, 398.1603).

Compound **5**: Colorless solid. UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 325 (4.42), 292 (3.95), 220 (4.10). IR $\nu_{max}$=3550, 3580, 2938, 1655, 885, 815 $cm^{-1}$. $^1$H-NMR ($CDCl_3$+$CD_3OD$, 400 MHz) $\delta$: 7.36 (1H, d, *J*=15.4 Hz, H-7′), 6.94 (2H, d, *J*=8.2 Hz, H-2, 6), 6.92 (1H, d, *J*=1.8 Hz, H-2′), 6.90 (2H, d, *J*=8.3 Hz, H-3, 5), 6.83 (1H, dd, *J*=8.1, 1.7 Hz, H-6′), 6.79 (1H, d, *J*=8.9 Hz, H-5′), 6.15 (1H, d, *J*=15.5 Hz, H-8′), 3.75 (3H, s, OMe), 3.40 (2H, t, *J*=7.3 Hz, H-8), 2.63 (2H, t, *J*=7.4 Hz, H-7), 1.13 (2H, t, *J*=5.2 Hz, H-1″), 1.05—0.85 (4H, m, H-2″, 3″), 0.75 (3H, t, *J*=7.5 Hz, H-4″). $^{13}$C-NMR ($CDCl_3$+$CD_3OD$) $\delta$: 168.5 (C=O), 148.9 (C-3′), 148.5 (C-4′), 141.5 (C-7′), 135.4 (C-1), 135.4 (C-4), 129.9 (C-6), 129.9 (C-2), 129.9 (C-3), 129.9 (C-5), 127.7 (C-1′), 122.7 (C-6′), 118 (C-8′), 115.8 (C-5′), 111.1 (C-2′), 56.3 (OMe), 40.9 (C-8), 34.6 (C-7), 29.6 (C-1″), 29.4 (C-2″), 28.5 (C-3″), 13.8 (C-4″). MS *m/z*: 353 $[M]^+$ (10), 204 (40), 177 (100), 176 (40), 150 (23), 136 (65). HR-MS *m/z* 353.1992 (Calcd for $C_{22}H_{27}NO_3$, 353.1990).

Compound **5a**: Solid, $^1$H-NMR ($CDCl_3$, 400 MHz) $\delta$: 7.35 (1H, d), 6.94 (2H, d), 6.89 (2H, d), 6.87 (1H, d), 6.85 (1H, dd), 6.79 (1H, d), 6.15 (1H, d), 3.80 (3H, s), 3.50 (2H, t), 2.71 (2H, t), 2.25 (3H, s), 1.14 (2H, t), 1.06—0.89 (4H, m), 0.77 (3H, t). HR-FAB-MS *m/z* 397.3142 $[M+H]^+$ (Calcd for $C_{24}H_{31}NO_4$, 397.3170).

**Assay of $\alpha$-Glucosidase Inhibition** Enzyme inhibitory activity against $\alpha$-glucosidase type VI (Sigma G6136) was observed spectrophotometrically at pH 6.8 and at 37 °C using 0.7 mM *p*-nitrophenyl $\alpha$-D-glucopyranoside (PNP-G) as a substrate and 0.017 units/ml enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl and 0.3 mM 1-deoxynojirimycin was used as a positive control ($IC_{50}$=0.3 mM). The absorption increase at 400 nm due to hydrolysis of PNP-G by $\alpha$-glucosidase was monitored continuously with a spectrophotometer (Molecular Devices, U.S.A.).

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