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New Enzyme Inhibitory Constituents from *Tribulus longipetalus*

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Abstract: Normal and reversed phase chromatographic purification of the chloroform soluble fraction of the methanolic extract of *Tribulus longipetalus* led to the isolation of a new tyramine amide, longipetalamide (**1**), two new benzocoumarins, longipetalasin A (8-*n*-propyl-*threo*-1′*S*,2′*S*-dihydroxy-5-methoxy-5a,9a-benzocoumarin; **2**) and B (8-*n*-propyl-*threo*-1′*S*,2′*S*-dihydroxy-5,10-dimethoxy-5a,9a-benzocoumarin; **3**) together with 1,2,3-propantriyl trioleate (**4**), crotamide A (**5**), stigmasterol (**6**), (25*S*)-5*α*-furustan-22-methoxy-3*β*,26-diol (**7**), neotigogenin (**8**), tigogenin (**9**), methyl 4-hydroxyphenyl acetate (**10**) and 2-*O*-methylinositol (**11**). All the isolates (**1-11**) were characterized by using UV, IR, 1D- (^{1}H and ^{13}C), 2D-NMR (HSQC, HMBC, COSY) spectroscopy, mass spectrometry (EI-MS, HR-EI-MS, FAB-MS, HR-FAB-MS) and in comparison with the data reported in literature. The compounds **1-11** were evaluated for their enzyme inhibition studies against *α*-glucosidase, lipoxygenase (LOX), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes and found that **2** and **3** were the significant inhibitors of enzyme *α*-glucosidase with IC_{50} values 94.17 ± 0.09 and 85.65 ± 0.08 µM, respectively.

Keywords: *Tribulus longipetalus*; Secondary Metabolites; Isolation; Characterization; Enzyme Inhibition.

1. Introduction

Tribulus longipetalus is an annual or biennial prostate herb, found in dry sandy soils in warm temperate zones of Egypt and Asia. The fruit of this plant is used for the treatment of urinary disorders and cough [1-2]. *T. terresteris* is a famous pharmaceutical herb in producing sex enhancing chemicals like Tribestan, Vitanone, and Tribusaponin [3]. It also possesses aphrodiasic properties, due to the diuretic action of its fruits and found highly beneficial for urolithiasis [4]. *T. terresteris* also promotes digestive power, and useful in cough, diabetes, piles, rheumatism and heart diseases [5]. Literature survey revealed the presence of spirostan, furostane and cholestane steroidal saponins together with flavonol glycosides, ligninamides and alkaloids [6-7] in different *Tribulus* species [2,8-10].

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Previously, we have reported steroidal saponins from *T. longipetalis* [11]. Herein, we report the isolation and structure elucidation of a new amide named as longipetalamide (**1**), two new benzocoumarins, longipetalasins A (**2**) and B (**3**) together with eight known compounds (**4-11**).

2. Materials and Methods

2.1. General Experimental Procedures

Melting points were determined by Buchi 434 melting point apparatus. Optical rotations were measured on a Jasco DIP-360 polarimeter. UV spectra were obtained in methanol on a U-3200 Schimadzu UV-240 spectrophotometer. Infrared (IR) spectra were recorded on a Shimadzu 460 spectrometer. ^{1}H- (400, 500 MHz), ^{13}C-NMR (100, 125 MHz) and 2D-NMR (HMQC, HMBC and COSY; 400, 500 MHz) spectra were recorded on a Bruker spectrometer. The chemical shift values (δ) are reported in ppm, and the coupling constants (J) are in Hz. EI-MS, FAB-MS, HR-EI-MS and HR-FAB-MS were recorded on a Finnigan (Varian MAT) JMS H×110 instrument with a data system and JMSA 500 mass spectrometers, respectively. Chromatographic separations were carried out using aluminum sheets pre-coated with silica gel 60 F_{254} (20×20 cm, 0.2 mm thick; E. Merck) for thin layer chromatography (TLC) and silica gel (230-400 mesh) for column chromatography. TLC plates were visualized under UV at 254 and 366 nm and by spraying with ceric sulfate solution and heating.

2.2. Plant Material

The whole plant of *Tribulus longipetalus* was collected from Cholistan Desert in May 2010 from District Bahawalpur (Punjab) Pakistan and was identified by Dr. Muhammad Arshad (late) Plant Taxonomist, Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan, where a voucher specimen (TL-CIDS-112/10) has been deposited.

2.3. Extraction and Isolation

The whole plant material of *T. longipetalus* (6 Kg) was dried under shade, ground and extracted thrice with Methanol (3 × 20 L). The methanolic extract was evaporated to dryness on rotary evaporators to obtain brownish gummy material (260 g) which was suspended in H_2O (1 L) and extracted with *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc) and *n*-butanol soluble fractions. The $CHCl_3$ soluble fraction (40 g) was subjected to column chromatography over silica and eluted with *n*-hexane, *n*-hexane-dichloromethane (DCM), dichloromethane (DCM), dichloromethane (DCM)-methanol and methanol in increasing order of polarity resulted into ten fractions (F-1 to F-10). These fractions were further purified using gradient elusion as the fraction F-1 was further purified using 15% DCM in *n*-hexane to get 1,2,3-propantriyl trioleate (**4**) (30 mg). Crotamide A (**5**) (30 mg) was purified by eluting fraction F-2 with 22% DCM in *n*-hexane. The fraction F-3 eluted with 25% DCM in *n*-hexane afforded longipetalamide (**1**) (30 mg). The fraction F-4 was eluted with 30% DCM in *n*-hexane to afford stigmasterol (**6**) (28 mg). (25*S*)-5α-Furustan-22-methoxy-3β,26-diol (**7**), (26 mg) was purified from fraction F-5 using 40% dichloromethane (DCM) in *n*-hexane. The fraction F-6 when eluted with 50% DCM in *n*-hexane led to the isolation of neotigogenin (**8**) (26 mg). Tigogenin (**9**) (25 mg) was obtained by eluting fraction F-7 with 75% DCM in *n*-hexane. The fraction F-8 was further column chromatographed by eluting with 85% DCM in *n*-hexane to afford methyl 4-hydroxyphenyl acetate (**10**) (30 mg). The fraction F-9 was rechromatographed with 1% methanol in DCM led to the isolation of 2-*O*-methylinositol (**11**) (26 mg). The fraction F-10 obtained from 2% methanol in DCM showed a binary mixture on TLC which was further purified by CC using same solvent system resulted in longipetalasin A (**2**) (26 mg) from the head fractions and longipetalasin B (**3**) (28 mg) from the tail fractions, respectively.

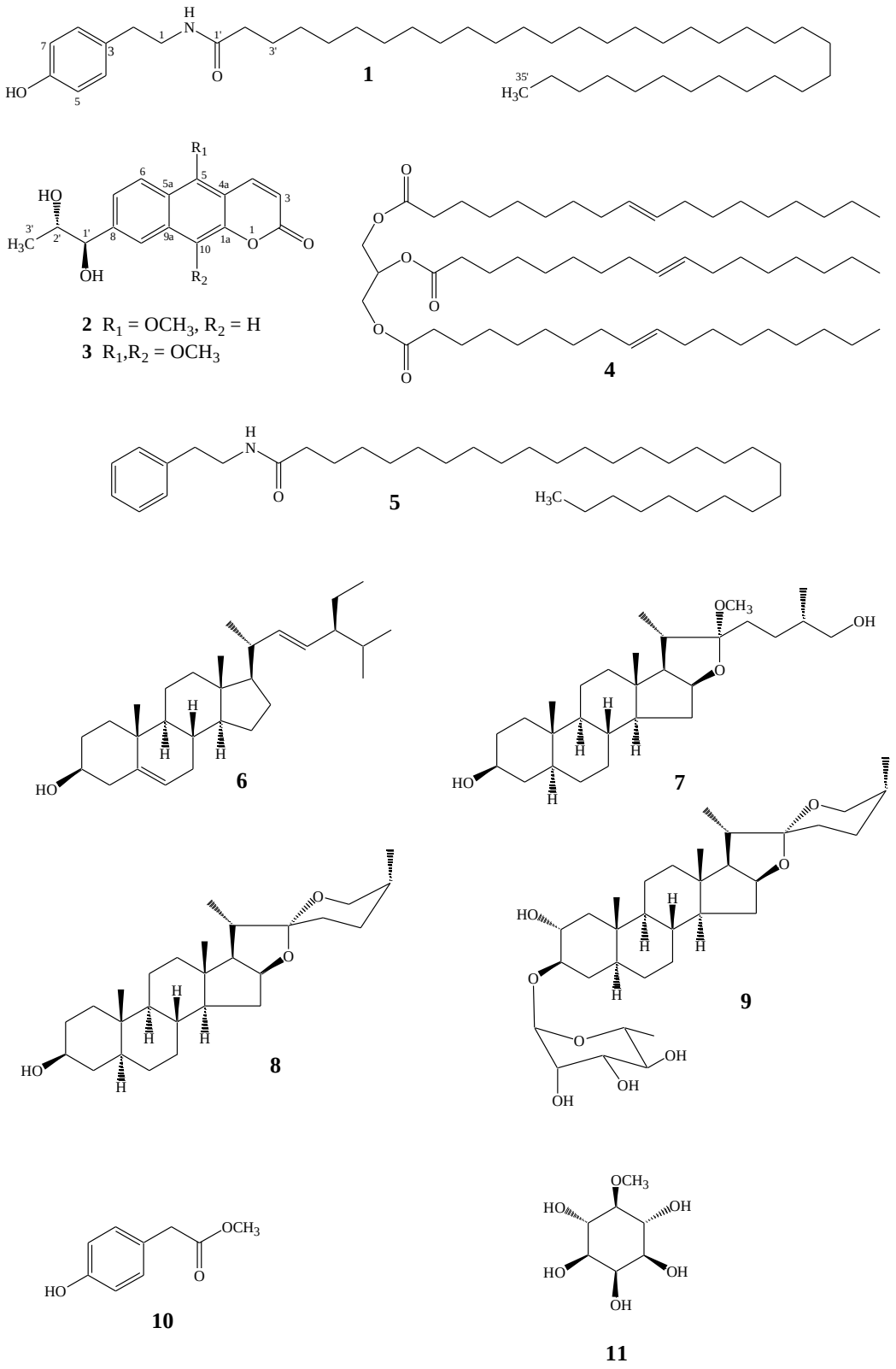

Figure 1. Structures of compounds (**1-11**) isolated from *Tribulus longipetalus.*

3. Results and Discussion

3.1. Structure elucidation

Compound **1** was isolated as a colorless amorphous powder. The UV spectrum showed the absorption bands at 200, 220, 289 and 317 nm, whereas, the IR spectrum showed characteristic absorption bands at 3400 and 1679 cm^{-1} for hydroxyl and amide functions, respectively. The HR-EI-MS spectrum showed molecular ion peak $[M]^+$ at *m/z* 641.6138 corresponding to the molecular formula $C_{43}H_{79}NO_2$ having five double bond equivalents (DBE). The major fragment ions were observed at *m/z* 612.5715 $[M\text{-}C_2H_5]^+$, 548.5765 $[M\text{-}C_6H_5O]^+$, 534.5608 $[M\text{-}C_7H_7O]^+$, 519.5375 $[M\text{-}C_8H_{10}O]^+$, due to the loss of terminal ethyl, *p*-hydroxyphenyl, *p*-hydroxybenzyl and *p*-hydroxyphenylethyl moieties, respectively. The ^{1}H-NMR spectrum of **1** (Table 1) showed a pair of doublets for an A^2B^2 system at δ 6.85 (2H, d, J = 8.5 Hz), and 6.61 (2H, d, J = 8.5 Hz) attested for a *para*-substituted benzene ring. The spectrum further showed resonances for two triplet methylenes at δ 3.25 (J = 7.0 Hz, H-1), and 2.59 (J = 7.0 Hz, H-2), which were found to be vicinal to each other through COSY spectrum. Another triplet methylene resonating at δ 1.97 (J = 7.5 Hz, H-2′) exhibited COSY correlation with a broad signal of several aliphatic methylenes at δ 1.42-1.11, which in turn was correlated with a triplet methyl at δ 0.73 (J = 7.0 Hz), confirming a long-chain aliphatic part in **1**. The ^{13}C-NMR spectrum of **1** (Table 1) showed the signals for an amide carbonyl carbon (δ 170.1), *p*-substituted benzene ring (δ 155.1, 130.0, 129.5, 115.2) two saturated methylenes (δ 34.5, 40.7) supporting to ^{1}H-NMR spectrum, confirmed the presence of tyramine nucleus [12]. The signals for aliphatic chain appeared at δ 36.4, 25.6, 21.9-31.7 and 14.0 [13]. All the assignments were done based on COSY, HSQC and HMBC experiments. The substitution and the linkages were confirmed by long range HMBC correlations in which H-1 (δ 3.25) correlated with carbonyl carbon (δ 170.1) and C-3 (δ 130.0) confirmed its attachment with amide function. Further HMBC and COSY correlations are shown in Table 1. Based on these evidences, compound **1** was characterized as N-(4-hydroxyphenethyl)-pentatriacontanamide and is named as longipetalamide which is a new addition in the list of natural products.

Compound **2** was isolated as colorless amorphous powder that displayed a molecular ion peak in the HR-EI-MS spectrum at *m/z* 300.0990, corresponding to the molecular formula $C_{17}H_{16}O_5$ with ten DBE. The IR spectrum showed absorption bands at 3340, 1718 and 1606 cm^{-1} for OH, carbonyl and aromatic moiety, respectively, whereas the UV bands were observed at 240, 318 and 353 nm attested for substituted aromatic system. The ^{1}H-NMR spectrum (Table 2) displayed six signals in the aromatic region at δ 8.02 (1H, d, J = 9.6 Hz), 6.89 (1H, d, J = 7.8 Hz), 6.79 (1H, d, J = 1.8 Hz), 6.49 (1H, d, J = 9.6 Hz), 6.18 (1H, s) and 6.09 (1H, dd, J = 7.8, 1.8 Hz). The ^{1}H-NMR spectrum showed typical signals at δ 8.02 and 6.49 with larger J values (9.6 Hz) attributed to a coumarin nucleus [14], whereas, the ABX splitting pattern of the three signals revealed a tri-substituted benzene ring in **2**. The number and splitting pattern of all the protons resonating in aromatic region revealed a benzocoumarin nature of **2** [15]. A 1,2-dihydroxypropyl moiety in **2** was identified due to the resonances of two oxygenated methines at δ 4.06 (1H, m), δ 4.64 (1H, d, J = 7.0 Hz) and a methyl at δ 1.24 (3H, d, J = 6.6 Hz), which were interconnected with one and other through COSY spectrum. The same spectrum further displayed a methoxyl signal at δ 3.89. The ^{13}C-NMR (BB and DEPT) (Table 2) data was fully supportive of mass and ^{1}H-NMR data as it signals for a substituted benzocoumarin nucleus at δ 160.2, 114.5, 139.9, 110.0, 145.9, 128.8, 113.9, 121.0, 127.4, 110.2, 125.2 and 108.9, dihydroxypropyl moiety at δ 79.9, 74.6 and 28.9, and methoxyl carbon at δ 56.5. All the C-H assignments were accomplished due to HMQC experiment, whereas, overall structural features could be defined due to HMBC analysis. The HMBC correlation of both H-3 (δ 6.49) and H-4 (δ 8.02) with C-2 (δ 160.2) indicated the presence of 2-pyrone nucleus [16]. The position of both methoxy and dihydroxy-*n*-propyl group was confirmed by HMBC correlations in which the methoxy signal (δ 3.89) found correlated with C-5 (δ 145.9) and the oxygenated methine H-1′ (δ 4.64) showed correlation with C-3′ (δ 28.9), C-7 (δ 121.0) and C-9 (δ 110.2).

The second oxygenated methine H-2′ (δ 4.06) showed HMBC correlation with C-3′ (δ 28.9) and C-8 (δ 127.4) and H-3′ (δ 1.24) showed HMBC correlation with C-2′ (δ 74.6) and C-1′ (δ 79.9). The remaining HMBC correlations are shown in Table 2. The relative stereochemistry of both the hydroxyl groups was deduced by ^{1}H- and ^{13}C-NMR (through chemical shifts and coupling constant) which found similar to the coupling constant of previously reported compounds [17-18] confirming **2** as 1′*S*,2′*S threo* geometry. The above discussed data led us to assign the structure **2** as 8-*n*-propyl-*threo*-1′*S*,2′*S*-dihydroxy-5-methoxy-5a,9a-benzocoumarin, which is a new phytochemical and is named as longipetalasin A.

The HR-EI-MS of compound **3** depicted the molecular formula as $C_{18}H_{18}O_6$ due to a molecular ion peak at *m/z* 330.1105, whereas, the NMR data (Table 2) was nearly super-imposable to the data of compound **2** telling the similar structural features. The main difference in data was the absence of an aromatic methine (δ_H 6.18; δ_C 108.9) and the resonance of an additional quaternary carbon at δ 142.0, which revealed that position-10 of benzocoumarin nucleus must be substituted. This deduction was further substantiated due to the resonance of an additional methoxyl moiety (δ_H 3.86, δ_C 56.0), which exhibited HMBC correlation with the C-10 (δ 142.0). The stereochemistry of **3** was done by same way as done for **2** and found similar and finally confirmed the structure **3** as 8-*n*-propyl-*threo*-1′*S*,2′*S*-dihydroxy-5,10-dimethoxy-5a,9a-benzocoumarin, named as longipetalasin B.

The structures of the known compounds; 1,2,3-propantriyl trioleate (**4**) **[**19], crotamide A (**5**) [20], stigmasterol (**6**) [21], (25*S*)-5α-furustane-22-methoxy-3β,26-diol (**7**) [22], neotigogenin (**8**) **[**22], tigogenin (**9**) [23], methyl 4-hydroxyphenyl acetate (**10**) [24] and 2-*O*-methyl inositol (**11**) [25] were confirmed by comparing their spectral data with the data reported in literature.

3.2. Enzyme Inhibition Studies of Compounds 1-11

The isolated compound **1-11** were evaluated for the enzyme inhibition studies against enzymes α-glucosidase, lipoxygenase, acetylcholinesterase and butyrylcholinesterase. The results (Table 3) showed that only **2** and **3** found to be significant inhibitors of enzyme α-glucosidase with IC_{50} values 94.17 ± 0.09 and 85.65 ± 0.08 μM, respectively.

3.3. Spectroscopic Data of Compounds 1-2

*3.3.1. Longipetalamide **1***

White amorphous powder (30 mg). UV (CH_3OH) λ_{max} (nm): 200 (2.6), 220 (3.1), 289 (3.9), 317 (4.01). IR (KBr) υ_{max} (cm^{-1}): 3400, 3313, 1679, 1505. ^{1}H- and ^{13}C-NMR, see Table 1. HR-EI-MS: *m/z* 641.6138 (calcd. for $C_{43}H_{79}NO_2$, 641.6110).

*3.3.2. Longipetalasin A [8-n-propyl-threo-1′S,2′S-dihydroxy-5-methoxy-5a,9a-benzocoumarin] **2***

White amorphous powder (26 mg). UV (CH_3OH) λ_{max} (nm): 240 (4.1), 318 (3.9), 353 (3.6). IR (KBr) υ_{max} (cm^{-1}): 3340, 1718, 1606. ^{1}H- and ^{13}C-NMR data, see Table 2. HR-EI-MS: *m/z* = 300.0990 (calcd. for $C_{17}H_{16}O_5$, 300.997).

*3.3.3. Longipetalasin B [8-n-propyl-threo-1′S,2′S-dihydroxy-5,10-dimethoxy-5a,9a-benzocoumarin] **3***

White amorphous powder (28 mg). UV (CH_3OH) λ_{max} (nm): 240 (3.9), 318 (3.7), 353 (3.2). IR (KBr) υ_{max} (cm^{-1}): 3340, 1718, 1606. ^{1}H- and ^{13}C-NMR data, see Table 2. HR-EI-MS: *m/z* 330.1105 (calcd. for $C_{18}H_{18}O_6$, 330.1103).

4. Enzyme Inhibition Assay

4.1. α-Glucosidase Inhibition Assay

The *α*-glucosidase inhibition assay was performed with slight modifications as done by Pierre *et al.* [26]. Total volume of 100 µL reaction mixture contained 70 µL 50 mM phosphate buffer, pH 6.8, 10 µL (0.5 mM) test compound, followed by the addition of 10 µL (0.0234 units, Sigma Inc.) enzyme. The contents were mixed, pre-incubated for 10 min at 37˚C and pre-read at 400 nm. The reaction was initiated by the addition of 10 µL of 0.5 mM substrate (*p*-nitrophenyl glucopyranoside, Sigma Inc.). After 30 min of incubation at 37˚C, absorbance of the yellow color produced due to the formation of *p*-nitrophenol was measured at 400 nm using Synergy HT (BioTek, USA) using 96-well microplate reader. Acarbose was used as positive control. The percent inhibition was calculated by the following equation.

$$\text{Inhibition (\%)} = (\text{abs. of control} - \text{abs. of test} / \text{abs. of control}) \times 100$$

IC_{50} values were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

4.2. Lipoxygenase Inhibition Assay

Lipoxygenase (LOX) activity was assayed according to the reported method [27] but with slight modifications. A total volume of 200 µL assay mixture contained 140 µL sodium phosphate buffer (100 mM, pH 8.0), 20 µL test compound and 15 µL (600U) purified lipoxygenase enzyme (Sigma, USA). The contents were mixed and pre-read at 234 nm and pre incubated for 10 minutes at 25˚C. The reaction was initiated by the addition of 25 µL substrate solution. The change in absorbance was observed after 6 min at 234 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All reactions were performed in triplicates. Baicalein (0.5 mM $well^{-1}$) was used as a positive control. The percentage inhibition was calculated by formula given below.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

IC_{50} values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

4.3. Acetylcholinesterase and Butyrylcholinesterase Inhibition Assay

The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activities was performed according to the method used by Ellman [28] with slight modifications. Total volume of the reaction mixture was 100 µL. It contained 60 µL Na_2HPO_4 buffer with concentration of 50 mM and pH 7.7. Ten µL test compound (0.5 mM $well^{-1}$) was added, followed by the addition of 10 µL (0.005 unit $well^{-1}$) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 min at 37˚C. The reaction was initiated by the addition of 10 µL of 0.5 mM $well^{-1}$ substrate (acetyl and butyryl thiocholine iodide), followed by the addition of 10 µL DTNB (0.5 mM $well^{-1}$). After 30 min of incubation at 37˚C, absorbance was measured at 405 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM $well^{-1}$) was used as a positive control. The percent inhibition was calculated by the help of following equation.

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Table 1. ^{1}H and ^{13}C NMR data, HMBC, and COSY correlations of **1** ($CHCl_3$, 400 and 100 MHz).

Position	δ_H (J = Hz)	δ_C	HMBC (H→C)	COSY (H→H)
1	3.25 (t, 7.0)	40.7	C-1′, C-2, C-3	H-1/H-2
2	2.59 (t, 7.0)	34.5	C-3, C-4, C-8	H-2/H-1
3	-	130.0	-	-
4	6.85 (d, 8.5)	129.5	C-2, C-6, C-8	H-4H-5
5	6.61 (d, 8.5)	115.2	C-3, C-6, C-7	H-5/H-4
6	-	155.1	-	-
7	6.61 (d, 8.5)	115.2	C-3, C-5, C-6	H-7/H-8
8	6.85 (d, 8.5)	129.5	C-2, C-4,C-6	H-8/H-7
1′	-	170.1	-	-
2′	1.97 (t, 7.5)	36.4	C-1′	H-2′/H-3′
3′	1.42 (m)	25.6	C-1′	H-3′H-2′
	1.46 (m)			
4′-34′	1.11 (br s)	29.1-31.7	-	-
Me-	0.73 (t, 7.0)	14.0	C-33′, C-34′	-

Table 2. ^{1}H and ^{13}C NMR data, and HMBC correlations of **2**, **3** ($CHCl_3$, 400 and 100 MHz).

	2			**3**		
Position	δ_H (J in Hz)	δ_C	HMBC (H→C)	δ_H (J in Hz)	δ_C	HMBC (H→C)
1	-	-	-	-	-	-
2	-	160.2	-	-	160.9	-
3	6.49 (d, 9.6)	114.5	C-2, C-4a	6.36 (d, 9.6)	114.6	C-2, C-4a
4	8.02 (d, 9.6)	139.9	C-2, C-5, C-1a	8.02 (d, 9.6)	140.4	C-2, C-5, C-1a
4a	-	110.0	-	-	110.2	-
5	-	145.9	-	-	146.9	-
5a	-	128.8	-	-	128.1	-
6	6.89 (d, 7.8)	113.9	C-8, C-5, C-9a	6.90 (d, 7.8)	114.8	C-8, C-5, C-9a
7	6.09 (dd, 7.8, 1.8)	121.0	C-9, C-1′, C-5a	6.29 (dd, 7.8, 1.8)	120.9	C-9, C-1′, C-5a
8	-	127.4	-	-	127.9	-
9	6.79 (d, 1.8)	110.2	C-7, C-1′, C-10	6.83 (d, 1.8)	109.5	C-7, C-1′, C-10
9a	-	125.2	-	-	128.0	-
10	6.18 (s)	108.9	C-9, C-5a, C-9a	-	142.0	-
1a	-	138.9	-	-	138.4	-
-OMe	3.89 (s)	56.5	C-5	3.90 (s)	56.1	C-5
-OMe	-	-	-	3.86 (s)	56.0	C-10
1′	4.64 (d, 7.0)	79.9	C-3′, C-7, C-9	4.61 (d, 7.8)	81.6	C-3′, C-7, C-9
2′	4.06 (m)	74.6	C-3′, C-8	4.16 (m)	74.5	C-3′, C-8
3′	1.24 (d, 6.6)	28.9	C-1′, C-2′	1.26 (d, 6.6)	29.7	C-1′, C-2′

Table 3. *α*-Glucosidase, LOX, AChE, BChE inhibition studies of compounds **1-11.**

S. No	Conc.	*α*-glucosidase	IC_{50} (µM)	LOX	IC_{50} (µM)	AChE	IC_{50} (µM)	BChE	IC_{50} (µM)
1	0.5	10.34 ± 1.25	-	40.16 ±1.36	-	4.91 ± 0.11	-	20.55 ± 0.14	-
2	0.5	98.19 ± 0.45	94.17 ± 0.09	36.75 ±0.48	-	20.31 ± 0.35	-	28.14 ± 0.45	-
3	0.5	97.53 ± 0.37	85.65 ± 0.08	38.89 ±0.96	-	24.72 ± 0.41	-	19.45 ± 0.26	-
4	0.5	42.37±0.04	277.7±0.003	NA	NA	NA	NA	NA	NA
5	0.5	13.17 ± 0.34	-	36.61 ±1.04	-	11.82 ± 0.44	-	26.35 ± 0.31	21.21 ± 0.81
6	0.5	42.37 ± 0.04	277.7 ± 0.03						
7	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	0.5	6.44±0.45	-	67.25 ±1.29	163.16±1.22	9.85 ± 0.18	-	17.42 ± 0.25	-
11	0.5	8.23 ± 0.45	-	51.59 ±1.19	<400	31.28 ± 0.41	>500	30.18 ± 0.43	>500
Acarbose	0.5	92.23 ± 0.14	38.25 ± 0.12	-	-	-	-	-	-
Baicalein	0.5	-	-	93.79 ±1.27	22.4 ± 1.3	-	-	-	-
Eserine	0.5	-	-	-	-	91.27 ± 1.17	0.04 ± 0.0001	82.82 ± 1.09	0.85 ±0.0001

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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