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Synthesis of O-Substituted Derivatives of Tetrahydrocannabinol with Potential Butyrylcholinesterase Activity

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Tetrahydrocannabinol is the major active constituent of *cannabis sativa* and has many medicinal properties. In present work, a pure compound, tetrahydrocannabinol **(1)** was isolated from the dark viscous oil of *cannabis sativa* by column chromatography using different solvent systems. The purified tetrahydrocannabinol compound **(1)** on further treatment with different electrophiles (**2a-f**) in the presence of sodium hydride and *N,N*-dimethyl formamide furnished O-substituted derivatives of tetrahydrocannabinol (**3a-f**). The structures of all the synthesized compounds have been deduced from their spectral (^{1}H NMR, IR and EI-MS) data. Further, all these compounds were screened against butyrylcholinesterase enzyme and found that these compounds displayed activity to varying degree.

Key Words: Tetrahydrocannabinol, Column chromatography, Butyrylcholinesterase, IR, EI-MS, ^{1}H NMR.

INTRODUCTION

Cannabis sativa L., (marijuana) belongs to the family of *Moraceae*. This plant is indigenous to South East Asia, Africa and North America. The female plants are more preferred and used as they are rich in glandular trichomes containing resins. The possession, commercial sale, distribution and purchase of *cannabis* are prohibited under the drug act 1971. It is scheduled under the "Class B" of drugs that are abused. The prepared *cannabis* is deteriorated by oxidation if stored for more than 1 year and loses its grandness in the drug market. So it is dried and stored in well closed containers. *Cannabis* contains over 60 compounds that are collectively known as, cannabinoids. The major resin components include: tetrahydrocannabinol, cannabinol, cannabidiol, cannabidinolic acid, cannabichromene and cannabigerol[1-3] (Fig. 1)

Tetrahydrocannabinol (the main active constituent of *cannabis*) has been in use in many ailments and medical conditions for a long period. It is used in the field of medicine as hypnotic, anti-consulvant and an analgesic and also in anxiety and cough. It is used to relief vomiting and nausea in chemotherapy patients and as an appetite stimulant in AIDS patients. With long term usage of tetrahydrocannabinol, it has many adverse effects and is addictive in nature. It can cause complex disorders in patients like attention defects, loss of concentration and unable to perform high ranked tasks. It also affects the brain by altering the function of CNS system producing excitement followed by hallucinations and subsequent sleep. Chronic users may develop tolerance and withdrawal symptoms including sleeplessness, depression, anxiety, increased tension, agitation, anorexia and muscular tremors[4-15].

Butyryl cholinesterase (BChE, EC 3.1.1.8) constitutes a group of enzymes including serine hydrolazes. The different particularities for substrates and inhibitors for this enzyme are because of the differences in amino acid residues of the active sites of BChE. This enzyme system is credit worthy for the termination of acetylcholine at cholinergic synapses which are the basic components of cholinergic brain synapses and neuromuscular junctions. The main function of BChE is to catalyze hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses. It's now known that BChE is present in Alzheimer's plaques in non-negligibly greater quantities than in the normal age associated with non-dementia of brains. Cholinesterase inhibitors enhance the quantity of acetylcholine useable for neuronal and neuromuscular transmission due to their ability, reversibly or irreversibly. Thus the seeking new cholinesterase inhibitors is believed an essential and continuing **Scheme-I** to inaugurate new drug candidates for the treatment of Alzheimer's syndrome and other related diseases[16-19].

In protraction of our previous synthetic work[20], the present research work was an effective effort to synthesize *O*-substituted

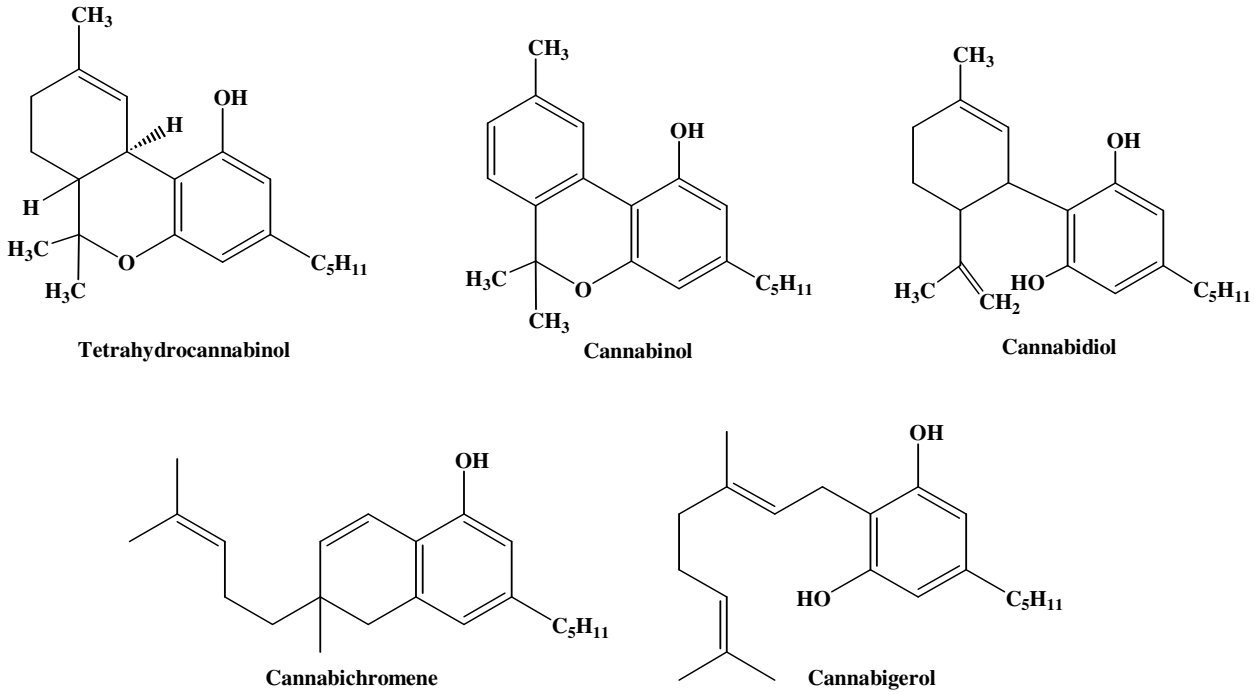
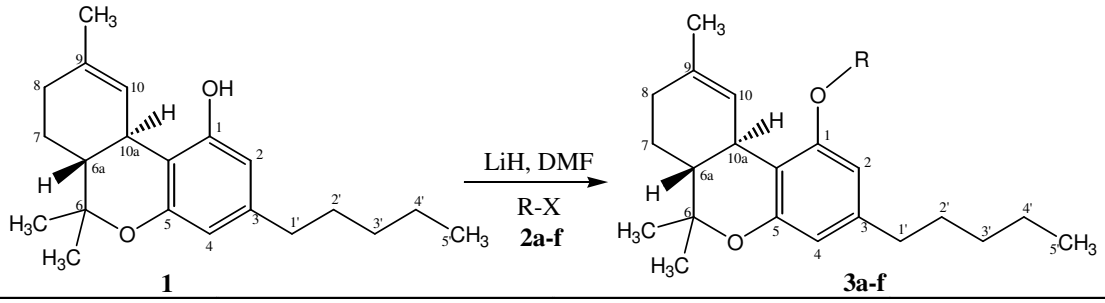
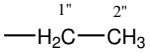
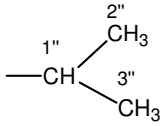
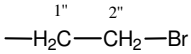
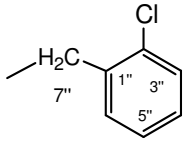
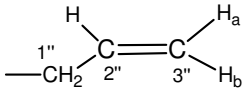
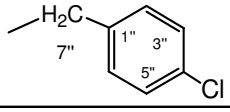


Fig. 1



Compound No.	-R	Compound No.	-R
2a		**2d**	
2b		**2e**	
2c		**2f**	

Scheme-I: Synthesis of *O*-substituted derivatives of tetrahydrocannabinol (THC)

derivatives of tetrahydrocannabinol by alkyl/aralkyl halides with an objective to produce new drug candidates that would have enhanced substantial biological and medicinal activities and can help to control various ailments.

EXPERIMENTAL

Purity of the compounds was checked by thin layer chromatography (TLC) with solvent systems using EtOAc and *n*-hexane on aluminum sheets precoated with silica gel 60 F_{254} (20 cm × 20 cm, 0.2 mm thick; E-Merck). Visualization of the TLC plates was carried out under UV at 254 and 366 nm and also by spraying with ceric sulfate solution (with heating). The IR spectra were recorded in KBr pellet method on a Jasco-320-A spectrophotometer (wave number in cm^{-1}). ^1H NMR spectra were recorded in CD_3OD on a Bruker spectrometers operating at 400 MHz. The chemical shift values are reported in ppm (δ) units taking TMS as reference standard and the coupling constants (*J*) are in Hz. Mass spectra (EIMS) were recorded on a JMS-HX-110 spectrometer. Alkyl/aralkyl halides were purchased from Merck and Alfa Aeser through

local suppliers. All the other used solvents were of analytical grade.

Isolation of tetrahydrocannabinol by column chromatography: The dark viscous oil of *cannabis sativa* was obtained (50 g) from Punjab Forensic Agency, Lahore, Pakistan for this study. The dark viscous oil (10 g) was dissolved in methanol and then filtered it by using the filter paper in order to remove all the suspended impurities. The filtrate then used to form slurry by using small amount of flash silica. The slurry was then subjected to column chromatography over flash silica using *n*-hexane, *n*-hexane-EtOAc and EtOAc as eluents in increasing order of polarity. The fraction obtained from *n*-hexane-EtOAc (8:2) was rechromatographed over flash silica using *n*-hexane-$CHCl_3$ (8.5:1.5) to afford the title compound, tetrahydrocannabinol (2 g).

General procedure for the synthesis of *O*-alky substituted derivatives (3a-f): Tetrahydrocannabinol (0.064 mol, **1**) was taken in round bottom flask and N,N-dimethyl formamide (around 5 mL) and sodium hydride (0.01g, 0.42 mmol) were added in it at room temperature. The reaction mixture was stirred for 0.5 h and then the corresponding electrophiles (0.064 mol, **2a-f**) were added into the mixture. The reaction mass was further stirred and monitored through thin layer chromatographic (TLC) plate using ethyl acetate and *n*-hexane (80:20) as mobile phase. After completion of the reaction, the reaction mixture was quenched with cold water (100 mL). The received solid was filtered, washed with distilled water and dried to yield the corresponding *O*-substituted derivatives of tetrahydrocannabinol (**3a-f**)[20-21].

Butyrylcholinesterse assay: The BChE inhibition was accomplished according to the reported method[22] with some differences. Volume of the reaction contents was 100 µL with 60 µL buffer (Na_2HPO_4, 50 mM with pH 7.7), 10 µL test compound (0.5 mM $well^{-1}$) and 10 µL BChE (0.5 unit $well^{-1}$). The reaction contents were fused and pre-read at 405 nm; also pre-incubated for 10 min at 37 ºC. The reaction was originated by the addition of 10 µL substrate (butyrylthiocholine bromide, 0.5 mM $well^{-1}$) followed by 10 µL DTNB (0.5 mM $well^{-1}$). After 15 min of incubation at 37 ºC, absorbance was measured at 405 nm. Synergy HT (Bio Tek, 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. Inhibition (%) = (Abs of control – Abs of test comp/Abs of control) × 100. IC_{50} values were designed using EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

IC_{50} values were determined by serial dilution of the compounds from 0.5 mM to 0.25, 0.125, 0.0625, 0.03125, 0.015625 mM. IC_{50} value was calculated from the graph, the concentration at which the enzyme inhibition was 50 %. Values are mean of 3 independent experiments.

Statistical analysis: All the measurements were done in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results are presented as mean ± sem.

Characterization of the synthesized compounds

(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromen-1-ol (1): Sticky brownish black solid; Yield: 63 %; m.f. $C_{21}H_{30}O_2$; m.w. 314 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3350 (O-H, stretching), 3035 (C-H, stretching of aromatic ring), 2919 (-CH_2-, stretching), 1543 (C=C, stretching of aromatic ring), 1225 (C-O-C, stretching of ring); ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 6.20 (s, 1H, H-2), 6.11 (s, 1H, H-4), 5.54 (br.s, 1H, H-10), 4.54 (m, 1H, H-10a), 2.44 (t, *J* = 8.0 Hz, 2H, CH_2-1'), 1.77 (s, 3H, CH_3-9), 1.65-1.76 (m, *J* =6.4 Hz, 1H, H-6a), 1.58-1.64 (m, 2H, CH_2-8), 1.53-1.55 (m, 2H, H-2'), 1.38 (s, 6H, 2CH_3-6), 1.23-1.41 (m, 4H, CH_2-3', CH_2-4'), 0.84 (t, *J* = 14.0 Hz, 3H, CH_3-5'), 0.78-1.07 (m, 2H, CH_2-7); EIMS**:** *m/z* 314 $[M]^+$, 299 $[M\text{-}CH_3]^+$, 272 $[M\text{-}C_3H_6]^+$, 232 $[M\text{-}C_6H_{10}]^+$, 54 $[C_4H_6]^+$.

(6aR,10aR)-1-Ethoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromene (3a): Yield: 67 %; m.f. $C_{23}H_{34}O_2$; m.w. 342 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3030 (C-H, stretching of aromatic ring), 2923 (-CH_2-, stretching), 1548 (C=C, stretching of aromatic ring), 1229 (C-O-C, stretching of ring); ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 6.25 (s, 1H, H-2), 6.18 (s, 1H, H-4), 5.53 (br.s, 1H, H-10), 4.51 (s, 1H, H-10a), 4.36 (q, *J* = 7.2 Hz, 2H, CH_2-1''), 2.41 (t, *J* = 8.0 Hz, 2H, CH_2-1'), 1.75 (s, 3H, CH_3-9), 1.63-1.74 (m, *J* = 6.4 Hz, 1H, H-6a), 1.56-1.66 (m, 2H, CH_2-8), 1.51-1.54 (m, 2H, H-2'), 1.38 (s, 6H, 2CH_3-6), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3-2''), 1.23-1.41 (m, 4H, CH_2-3', CH_2-4'), 0.85 (t, *J* = 14.0 Hz, 3H, CH_3-5'), 0.78-1.07 (m, 2H, CH_2-7); EIMS**:** *m/z* 342 $[M]^+$, 327 $[M\text{-}CH_3]^+$, 300 $[M\text{-}C_3H_6]^+$, 297 $[M\text{-}OC_2H_5]^+$, 260 $[M\text{-}C_6H_{10}]^+$, 54 $[C_4H_6]^+$.

(6aR,10aR)-1-(2-Bromoethoxy)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c] chromene (3b): Yield: 59 %; m.f. $C_{23}H_{33}BrO_2$; m.w. 421 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3033 (C-H, stretching of aromatic ring), 2917 (-CH_2-, stretching), 1541 (C=C, stretching of aromatic ring), 1223 (C-O-C, stretching of ring), 510 (C-Br, stretching); ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 6.23 (s, 1H, H-2), 6.17 (s, 1H, H-4), 5.51 (br.s, 1H, H-10), 4.49 (s, 1H, H-10a), 4.33 (q, *J* = 7.2 Hz, 2H, CH_2-1''), 3.84 (t, *J* = 7.2 Hz, 2H, CH_2-1''), 3.53 (t, *J* = 7.2 Hz, 2H, CH_2-2''), 1.76 (s, 3H, CH_3-9), 1.63-1.75 (m, 1H, H-6a), 1.53-1.62 (m, 2H, CH_2-8), 1.52-1.55 (m, 2H, H-2'), 1.39 (s, 6H, 2CH_3-6), 1.25-1.38 (m, 4H, CH_2-3', CH_2-4'), 0.81 (t, *J* = 14.0 Hz, 3H, CH_3-5'), 0.79-1.10 (m, 2H, CH_2-7); EIMS: *m/z* 423 $[M + 2]^+$, 421 $[M]^+$, 406 $[M\text{-}CH_3]^+$, 379 $[M\text{-}C_3H_6]^+$, 339 $[M\text{-}C_6H_{10}]^+$, 298 $[M\text{-}OC_2H_4Br]^+$, 54 $[C_4H_6]^+$.

(6aR,10aR)-1-(Allyloxy)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c] chromene (3c): Yield: 63 %; m.f. $C_{24}H_{34}O_2$; m.w. 354 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3036 (C-H, stretching of aromatic ring), 2920 (-CH_2-, stretching), 1544 (C=C, stretching of aromatic ring), 1226 (C-O-C, stretching of ring); ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 6.24 (s, 1H, H-2), 6.18 (s, 1H, H-4), 5.99 (m, 1H, CH-allylic-2''), 5.52 (br.s, 1H, H-10), 5.33 (dd, *J* = 15.9, 1.2 Hz, 1H, H_a-3''), 5.17 (dd, *J* = 15.9, 1.2 Hz, 1H, H_b-3''), 4.47 (s, 1H, H-10a), 3.80 (d, *J* = 7.2 Hz, 2H, H-1''), 1.74 (s, 3H, CH_3-9), 1.61-1.76 (m, *J* = 6.4 Hz, 1H, H-6a), 1.53-1.57 (m, 2H, H-2'), 1.51-1.60 (m, 2H, CH_2-8), 1.40 (s, 6H, 2CH_3-6), 1.24-1.36 (m, 4H, CH_2-3', CH_2-4'), 0.82 (t, *J* = 14.0 Hz, 3H, CH_3-5'), 0.80-1.11 (m, 2H, CH_2-7); EIMS: *m/z* 354 $[M]^+$, 339 $[M\text{-}CH_3]^+$, 312 $[M\text{-}C_3H_6]^+$, 297 $[M\text{-}OC_3H_5]^+$, 272 $[M\text{-}C_6H_{10}]^+$, 54 $[C_4H_6]^+$.

(6aR,10aR)-1-Isopropoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c] chromene (3d): Yield: 72 %; m.f. $C_{24}H_{36}O_2$; m.w. 356 g/mol; IR (KBr, ν_{max}, cm^{-1}):

3037 (C-H, stretching of aromatic ring), 2921 (-CH_2-, stretching), 1546 (C=C, stretching of aromatic ring), 1228 (C-O-C, stretching of ring); ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 6.20 (s, 1H, H-2), 6.15 (s, 1H, H-4), 5.54 (br.s, 1H, H-10), 4.45 (s, 1H, H-10a), 2.88 (m, 1H, H-1''), 1.75 (s, 3H, CH_3-9), 1.60-1.74 (m, *J* = 6.4 Hz, 1H, H-6a), 1.54-1.56 (m, 2H, H-2'), 1.52-1.62 (m, 2H, CH_2-8), 1.42 (s, 6H, 2CH_3-6), 1.25-1.37 (m, 4H, CH_2-3', CH_2-4'), 1.13 (d, *J* = 6.0 Hz 6H, CH_3-2'', CH_3-3''), 0.83-1.12 (m, 2H, CH_2-7), 0.81 (t, *J* = 14.0 Hz, 3H, CH_3-5'); EIMS**:** *m/z* 356 $[M]^+$, 341 $[M\text{-}CH_3]^+$, 314 $[M\text{-}C_3H_6]^+$, 297 $[M\text{-}OC_3H_7]^+$, 274 $[M\text{-}C_6H_{10}]^+$, 54 $[C_4H_6]^+$.

(6aR,10aR)-1-(2-Chlorobenzyloxy)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromene (3e): Yield: 57 %; m.f. $C_{28}H_{35}O_2Cl$; m.w. 438.5 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3031 (C-H, stretching of aromatic ring), 2916 (-CH_2-, stretching), 1541 (C=C, stretching of aromatic ring), 1221 (C-O-C, stretching of ring), 720 (C-Cl, stretching); ^{1}H NMR (400 MHz, CD_3OD, ppm): δ 7.38 (dd, *J* = 6.0, 1.5 Hz, 1H, H-3''), 7.25 (m, 3H, H-4'' to H-6''), 6.24 (s, 1H, H-2), 6.18 (s, 1H, H-4), 5.52 (br.s, 1H, H-10), 4.51 (s, 2H, CH_2-7''), 4.47 (s, 1H, H-10a), 1.74 (s, 3H, CH_3-9), 1.61-1.76 (m, *J* = 6.4 Hz, 1H, H-6a), 1.53-1.57 (m, 2H, H-2'), 1.51-1.60 (m, 2H, CH_2-8), 1.40 (s, 6H, 2CH_3-6), 1.24-1.36 (m, 4H, CH_2-3', CH_2-4'), 0.82 (t, *J* = 14.0 Hz, 3H, CH_3-5'), 0.80-1.11 (m, 2H, CH_2-7); EIMS**:** *m/z* 440 $[M+2]^+$, 438 $[M]^+$, 347 $[M\text{-}C_7H_7]^+$, 423 $[M\text{-}CH_3]^+$, 396 $[M\text{-}C_3H_6]^+$, 356 $[M\text{-}C_6H_{10}]^+$, 91 $[C_7H_7]^+$, 54 $[C_4H_6]^+$.

(6aR,10aR)-1-(4-Chlorobenzyloxy)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromene (3f): Yield: 57 %; m.f. $C_{28}H_{35}O_2Cl$; m.w. 438.5 g/mol; IR (KBr, ν_{max}, cm^{-1}): 3038 (C-H, stretching of aromatic ring), 2923 (-CH_2-, stretching), 1547 (C=C, stretching of aromatic ring), 1229 (C-O-C, stretching of ring), 718 (C-Cl, stretching); ^{1}H-NMR (400 MHz, CD_3OD, ppm): δ 7.34 (s, 1H, H-2), 7.21 (s, 1H, H-4), 6.27 (d, *J* = 8.4 Hz, 2H, H-3'', H-5''), 6.16 (d, *J* = 8.8 Hz 2H, H-2'', H-6''), 5.52 (br.s, 1H, H-10), 4.48 (s, 1H, H-10a), 1.72 (s, 3H, CH_3-9), 1.64-1.78 (m, *J* = 6.4 Hz, 1H, H-6a), 1.54-1.58 (m, 2H, H-2'), 1.53-1.63 (m, 2H, CH_2-8), 1.42 (s, 6H, 2CH_3-6), 1.25-1.37 (m, 4H, CH_2-3', CH_2-4'), 0.84-1.13 (m, 2H, CH_2-7), 0.83 (t, *J* = 14.0 Hz, 3H, CH_3-5'); EIMS**:** *m/z* 440 $[M+2]^+$, 438 $[M]^+$, 347 $[M\text{-}C_7H_7]^+$, 423 $[M\text{-}CH_3]^+$, 396 $[M\text{-}C_3H_6]^+$, 356 $[M\text{-}C_6H_{10}]^+$, 91 $[C_7H_7]^+$, 54 $[C_4H_6]^+$.

RESULTS AND DISCUSSION

In the undertaken research, a series of *O*-substituted derivatives of tetrahydrocannabinol (**3a-f**) were synthesized and screened against butyrylcholinesterase enzyme. These were developed by the coupling of tetrahydrocannabinol **(1)** with different electrophiles **(2a-f)** in the presence of sodium hydride and N,N-dimethyl formamide. Complete conversion was achieved within 30-60 min by stirring. The products were isolated by adding cold distilled water in the reaction mixture and filtering off the precipitated solid. In some cases, compound was taken out through solvent extraction method using chloroform or ethyl acetate. Structures of the parent compound and its *O*-substituted derivatives were confirmed by spectral data as described in experimental section. Parent compound **(1)** was separated as brown sticky solid. The molecular formula $C_{21}H_{30}O_2$ was established by EI-MS showing molecular ion peak 314 and by counting the number of protons in its ^{1}H NMR spectrum. The IR spectra showed absorption bands at 3350, 3035, 2919, 1543 and 1225 cm^{-1} which were assigned to O-H (stretching of hydroxyl group), C-H (aromatic stretching), -CH_2- (stretching of aliphatic), C=C (stretching of aromatic ring) and –C-O-C- (stretching of ring), respectively. The EI-MS gave peaks at *m/z* 299 and 271 which were attributed to the loss of methyl and propyl groups, respectively. A distinct peak at *m/z* 54 was due to butadiene cation. In the aromatic region of the ^{1}H NMR spectrum, the signals emerging at δ 6.20 (s, 1H, H-2) and 6.11 (s, 1H, H-4) showed the presence of benzene ring. In the aliphatic region, the signals appearing at δ 5.54 (s, 1H, H-10), 4.54 (s, 1H, H-10a), 1.77 (s, 3H, CH_3-9), 1.65-1.76 (m, 1H, H-6a), 1.58-1.64 (m, 2H, CH_2-8) and 0.78-1.07 (m, 2H, CH_2-7) demonstrated the presence of cyclohexene ring having a methyl group linked to it; the signals present at δ 1.38 (s, 6H, 2CH_3-6) revealed the bearing of two methyl groups at the same carbon linked with oxygen; the signals appeared at δ 2.44 (t, *J* = 8.0 Hz, 2H, CH_2-1'), 1.53-1.55 (m, 2H, H-2'), 1.23-1.41 (m, 4H, CH_2-3', CH_2-4'), 0.84 (t, *J* = 14.0 Hz, 3H, CH_3-5) revealed the presence of a pentyl group attached to aromatic ring. On the basis of above mentioned cumulative evidences, the structure of **(1)** was assigned (6aR,10aR)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromen-1-ol. Similarly, the structures of other compounds **(3a-f)** were characterized by ^{1}H NMR, IR and mass spectral data as described in experimental section.

Enzyme inhibition activity: The screening of all these synthesized compounds against butyrylcholinesterase (BChE) enzymes revealed that these molecules exhibited good inhibitory potential against butyrylcholinesterase, as evident from their IC_{50} values (Table-1). The parent compound **(1)** and (6aR,10aR)-1-(4-chlorobenzyloxy)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromene **(3f)** showed promising activity against butyrylcholinesterase enzyme having IC_{50} values 48.71 ± 0.25 and 59.31 ± 0.11 μmol/L, respectively, relative to Eserine, a reference standard with IC_{50} value of 0.85 ± 0.0001 μmol/L. These two compounds were the most active; the most probably because of the presence of alkyl/alkoxy/aralkyl groups in their structures. The compounds (6aR,10aR)-1-ethoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c] chromene **(3a)**, (6aR,10aR)-1-(2-bromo ethoxy)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromeneand **(3b)** and (6aR,10aR)-1-isopropoxy-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromene **(3d)** exhibited good inhibitory potential having IC_{50} values of 71.61 ± 0.08, 61.21 ± 0.31 and 73.31 ± 0.04 μmol/L, respectively. The only two compounds, (6aR,10aR)-1-(allyloxy)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromene **(3c)** and (6aR,10aR)-1-(2-chlorobenzyloxy)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromene **(3e)** showed weak inhibitory potential against butyrylcholinesterase enzyme. These compounds can further be exploited and their derivatives could be synthesized to get closer to IC_{50} value of the standard, eserine. In this way, the compounds could be potential target in the drug discovery and drug development program.

TABLE-1
BUTYRYLCHOLINESTERASE ENZYME INHIBITION ACTIVITY OF THC AND ITS DERIVATIVES

Sample code	BChE		
	Conc. (mM)	Inhibition (%)	IC_{50} (µmol)
1	0.50	88.08 ± 0.26	48.71 ± 0.25
3a	0.50	85.29 ± 0.18	71.61 ± 0.08
3b	0.50	86.85 ± 0.27	61.21 ± 0.31
3c	0.50	76.96 ± 0.11	95.21 ± 0.56
3d	0.50	79.21 ± 0.55	73.31 ± 0.04
3e	0.50	78.65 ± 0.39	112.31 ± 0.52
3f	0.50	84.95 ± 0.14	59.31 ± 0.11
Control (eserine)	0.25	82.82 ± 1.09	0.85 ± 0.0001

Note: 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). BChE = Butyrylcholinesterase.

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