Research Article

Synthesis and the Inhibitory Effects of Amino Acid Derivatives of 3-O-acetyl-11-keto-beta-boswellic Acid on Acetylcholinesterase

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ABSTRACT

Medicinal plants with anti-oxidation, anti-inflammation and anti-depression compounds have been administered traditionally in the treatment of some human diseases. Alzheimer's disease (AD) is one of the most debilitating diseases which have no definite cure. Recently, there is a tremendous surge in demand for natural non-steroidal drugs because of their established safety and efficacy through decades of usage by various cultures. In this study, we focused on designing and synthesis of four novel amino acids salts derivative of 3-O-acetyl-11-keto-beta-boswellic acid (AKBA) and their pharmaceutical effects evaluated by measuring of their acetyl cholinesterase (AChE) inhibitory activity. Therefore, at the first step, acetylation of boswellic acid (BA) using acetic anhydride and pyridine, following oxidation with chromium three oxides led to the formation of AKBA. Then AKBA were coupled with valine (Val), alanine (Ala), leucine (Leu) and isoleucine (Ile) in alkaline conditions of reaction. Experimental data showed that AKBA-Val and AKBA-Leu acted as appropriate inhibitors of AChE. The structure of all products in each step were characterized and confirmed by analytical and spectroscopic methods.

Keywords: 3-O-acetyl-11-keto-beta-boswellic acid, Alzheimer's disease, Acetyl cholinesterase, Amino acid, Boswellic acid.

INTRODUCTION

Olibanum gum, incense or frankincense, are the common names given to the oleogum resin that exudes from incisions in the bark of trees of Boswellia (Burseraceae). The pentacyclic triterpenic acids, named boswellic acids (BAs), are the main pharmacologically active ingredients of the resin of Boswellia, which have a chemical structure that closely resembles those steroids.[1] Several derivatives of boswellic acids have been synthesized and tested for a variety of biological and pharmacological activities (Figure 1).[2] Boswellic acid and its different derivatives were found to exhibit significant actions such as anti-arthritic[3], hepato-protective[4], and anti-viral[5] activities. Whereas, the 3-O-acetyl-11-keto-β-boswellic acid (AKβBA) as well as the relative structures were reported to have highly potent cytotoxicity on tumor cell lines in comparison with other triterpenes.[6] Moreover, its activity against ileitis, Crohn's disease,[7,8] ulcerative colitis, asthma, inflammatory diseases and Alzheimer's disease (AD) has been well documented.[9-11]

Therefore, the reaction between boswellic acid derivatives and organic amines leads to some compounds which are known to be more effective in healing the diseases such as cancer, joint pain, intestinal inflammation and AD. The studies showed that these compounds prevent the growth of cancer cells in vitro and they are also good examples of enzyme inhibitors for topoisomerases type I and IIα.[12] The anti-fever property of these compounds is directly related to the acidic feature as well as the changing of this structure into other functional groups.[8] Reduction of these salts leads to immediate emergence of free radicals as well as quick oxidation. Brain areas that are related to higher intellectual functions, especially the neocortex and hippocampus, are those most affected by the distinguished pathology of AD. This contains senile plaques created by the extracellular proteinaceous deposit of beta-amyloid, intracellular creation of neurofibrillary tangles (containing an unusual phosphorylated type of a microtubule associated protein, tau), and the lack of neuronal synapses and pyramidal neurons. The progression of the common symptomology of AD caused by these changes is described by progressive impairments of cognitive function and is often accompanied by behavioral disorders such as depression, aggression and wandering. Multiple studies have shown that clinical symptoms and the cognitive impairment in AD are correlated with the loss of cholinergic function. In more detail, deficits in the choline acetyl transferase (ChAT)[13], the enzyme that is responsible for the production of acetylcholine (ACh), reduced choline uptake[14] and ACh release along with the its recognized function in learning and memory have developed cholinergic hypothesis of AD.[15] Thus, it was suggested that deficiency of cholinergic neurons in the basal forebrain and the associated lack of cholinergic neurotransmission in the cerebral cortex and other

regions are the key factors in the deterioration in cognitive function observed in patients with AD.

The extracts from incense (olibanum) with boswellic acids as ingredient has been claimed to have therapeutics effects on a broad range of neurodegenerative conditions such as AD in addition to anti-inflammatory and anticancer activities, and the US patent has utilized the BA and incense extracts (olibanum) in a formulation for the prevention or treatment of AD.[16]

Regarding to these activities associated with BAs, we produced new organic salts using the combination of some amino acids including valine, alanine, leucine and isoleucine with AKBA, so that we can investigate possible improvements of its pharmaceutical effects in treatment of AD. This study is based on the reduction in the amounts of acetyl cholinergic neurons in the brain which was evaluated by detecting the inhibition of acetyl cholinesterase (AChE) enzyme using the synthesized amino acid salts.

MATERIALS AND METHODS

Instruments

IR spectra were recorded with a JASCO FT-IR 410 spectrophotometer by KBr pellet method. ^{1}H- and ^{13}C-NMR spectra were obtained with a Bruker 300 Avance spectrometer in DMSO as internal standard. MS spectra were determined with an HP-5973 network selective detector (electron impact, 70 Ev) instrument. CHN spectra were obtained on a PE 2400 Series II CHN/O Analyzer USA (Perkin Elmer) instrument.

Materials

All reagents in this study were purchased from Merck (Darmstadt, Germany), except BA that was provided by Sabinsa Corporation (Piscataway, NJ). All chemicals were reagent grade and advantaged without further purification.

Synthesis of 3-O-acetyl-11-keto-beta-boswellic acid (AKBA) (I)

1g of boswellic acid triterpene was added to a mixture of 1g of pyridine and 1g of acetic anhydride and the mixture was refluxed for 6h at 60-65 °C with stirring. The mixture was then allowed to be cooled at room temperature. Afterwards, 2.4g of acetic anhydride and 2.4g of acetic acid were added, and the reaction mixture stirred at the room temperature. At the same time, 0.64g of chromium three oxides was slowly added and reaction mixture vigorously stirred at 0-40 °C for 2h. The reaction mixture was poured into a distilled water mixture and after 24h resulted in formation of 3-O-acetyl-11-keto-beta-boswellic acid that was precipitated as a bright-russet color powder. The yield was then filtered and washed with distilled water and was applied for the next stages of the study.

Salt of the amino acid valine derivative of AKBA (KBA-Val) (II)

0.2 g of AKBA powder dispersed in 4ml of 95% aqueous methanol and was added to a solution containing 0.0252 g of amino acid valine in 0.6ml of water. The mixture stirred at room temperature for 15min and aqueous solution of potassium hydroxide (KOH) 20% was then added slowly to the reaction system, and stirred again at room temperature for 1h. Afterward, the mixture was evaporated under reduced pressure and dried in order to yield a bright-green color powder as compound (II). In the final product, the acetyl group of AKBA was hydrolyzed and converted to 11-keto-β-boswellic acid (KBA). We show this combination as KBA-Val.

Salt of the amino acid alanine derivative of AKBA (KBA-Ala) (III)

0.2 g of AKBA dispersed in 4ml of 95% aqueous methanol and was added to a solution containing 0.0252 g of amino acid alanine in 0.6ml of water. The mixture stirred at room temperature for 15 min and then aqueous solution of potassium hydroxide (KOH) 20% was gently added and stirred at room temperature for 1h. Finally, the mixture was evaporated under reduced pressure and dried in order to gain a high viscous green color matter as compound (III). In the final product, the acetyl group of AKBA was hydrolyzed and converted to KBA. We show this combination as KBA-Ala.

Salt of the amino acid leucine derivative of AKBA (KBA-Leu) (IV)

A solution containing 0.63g of amino acid leucine in 3 ml water was added to solution of AKBA (5g) in 100 ml of aqueous methanol 95% and stirred for 15 min at room temperature. Aqueous solution of KOH 20% was then added drop wise for 10 min and stirred continuously for 1 h. The solvent was evaporated under reduced pressure and dried to obtain compound (IV) as green color powder. In the final product, the acetyl group of AKBA was hydrolyzed and converted to KBA. We show this combination as KBA-Leu.

Salt of the amino acid isoleucine derivative of AKBA (KBA-Ileu) (V)

Isoleucine (0.63g) was dissolved in 3 ml of water and was added to a solution of AKBA (5g) in 95% aqueous methanol (100 mL). The mixture was stirred for 15 min and aqueous solution KOH 20% was then added slowly for 10 min. The mixture was stirred for 1 h and the solvent was evaporated under reduced pressure and dried to acquire a green color high viscous matter as compound (V). In the final product, the acetyl group of AKBA was hydrolyzed and converted to KBA. We show this combination as KBA-Ileu.

Animal

This study was carried out on extracted synaptosomes from sheep brain to investigate the effects of new

compounds on cholinergic synapses. The study was approved by the University of Tehran and Animal Sciences Research Institute of Iran. One adult male sheep (Afshari Persian) with 74.360 Kg body weight and good body condition was decapitated. The process of decapitation was carried out in the presence of Animal Sciences Research Institute representative and The Iranian Society for The Prevention of Cruelty to Animals agents in Ehsan slaughterhouse (Shahr-e-Ray, Iran). The carcass was delivered to Ehsan slaughterhouse. Sheep skull was cracked and split by an axe and the whole brain was removed. Then, cerebral cortex was separated and kept in sucrose 0.32 M to be used in synaptosome preparation step.

Preparation of synaptosomes

Synaptosomes were prepared by sucrose gradient centrifugation using the method of Dodd et al [17, 18]. The cerebral cortex of sheep brain was applied to prepare synaptosomes. Extracted cerebral cortex was minced and homogenized with Motor-Driven Potter Teflon-Glass Homogenizer at 800 rpm. The obtained homogenate was centrifuged at 3000 g for 30 min. Supernatant was loaded on top of sucrose 1.2 M. The sucrose gradients were centrifuged at 113000 g for 35 min. The soft middle white layer between the sucrose layers of 0.32 M and 1.2 M was then acquired and loaded on top of sucrose 0.8 M which was centrifuged at 113000 g for 35 min. The resulting pellet, containing synaptosomes, was dissolved in sucrose 0.32 M solutions. Finally, synaptosomes were stored at -20°C.

Transmission electron microscopy (TEM)

TEM micrographs were taken to verify morphology of synaptosomes. The synaptosome suspension was centrifuged at 9000 g for 30 min, the supernatant was acquired and the resulting pellet was fixed in 2.5% Glutaraldehyde for 1.5 h. The samples were rinsed twice by phosphate buffer for 5 min and were stained with 1% osmium tetroxide for 60 min. After dehydration by different concentrations of ethanol from 25% to 100%, the samples were mixed with agarose and sectioned by Richert Ultra microtome. Samples were stained with uranyl acetate and Pb citrate and were observed with a HU-12A electron microscope (Hitachi, Japan).

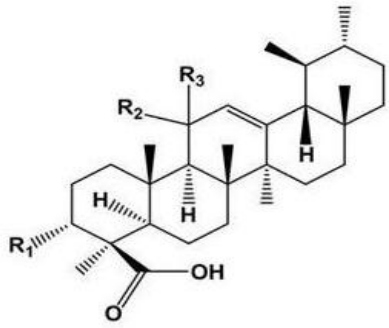
AChE activity assay

Specific activity of AChE was measured by Ellman method after incubation of 1 mg/ml of synaptosome suspension with 0.1 mg of each synthesized compounds [19]. This method is based on NTB^{2-} (2-nitro, 5-thiobenzoic acid) production and its absorption at 412nm. The samples consisting of synaptosomal suspension (200 µg protein), acetylthiocholine 1.2 mM and 5'-dithiobis-2-nitrobenzoic acid (DTNB 5) 1 mM were prepared in phosphate buffer 50 mM pH 7.2. The enzyme activity was assayed at 37ºC. The protein concentrations were determined for enzyme specific activity using Bradford method.[20]

RESULTS

FT-IR, GC-mass spectra and CHN analysis

All of the synthesized compounds 2-5 were characterized by FT-IR and mass spectroscopy and CHN analyzer. Infrared spectroscopy is an effective technique in order to identify the presence of certain functional groups in a molecule. An infrared spectrum represents a fingerprint of a sample with absorption peaks which corresponds to frequencies of vibrations between the bonds of atoms making up the material. Therefore, infrared spectroscopy can lead to qualitative analysis of every different kinds of material. Mass spectrometry is used to produce spectra of the masses of the atoms or molecules comprising a material. In this method, chemical structure of molecules is elucidated. CHN analysis is a form of elemental analysis that is based on combustion analysis where the sample is first fully combusted and then its elements (C, H and N) are analyzed. Hence, GC-mass and FT-IR spectroscopy and CHN analyzer were utilized to evaluate the structure of synthesized compounds (II-V) that were indicated below. In the case of AKBA synthesis which our study is based on that, ^{1}H- and ^{13}C-NMR spectroscopy were applied to elucidate its accurate synthesis.



R_1=OH,	R_2, R_3=H	β-Boswellic acid
R_1=OAc,	R_2, R_3=H	3-O-Acetyl- β-boswellic acid
R_1=OH,	R_2, R_3=O	11-Keto-β-boswellic acid
R_1=OAc,	R_2, R_3=O	3-O-Acetyl-11-Keto- β-boswellic acid

Figure 1: Chemical structures of boswellic acid.

3-O-acetyl-11-keto-beta-boswellic acid (AKBA) (I)

Yield, 42.3%; IR (KBr, cm^{-1}) υ: 1259.29 (-C-O-), 1379.50 (-CH_3), 1428.99 (-CH_2-) (attached to below shoulder area 1453.10), 1454.06-1618.95 (-C=C-), 1661.37 (-C=C-C-O-), 1706.02 (-COO^-), 1736.57 (-OCO-, ring) (below area 1620.88-1740.44), 2924.52 (-CH-), 3433.64 (-OH), above 3000 (-CH- of Pyridine); ^{1}H NMR (500 MH_Z, DMSO) δ: 0.91-2.03 (21H, H-23, H-25, H-26, H-27, H-28, H-29, H-30), 2.49 (6H of DMSO), 3.33 (3H, H-32), 3.59-4.56 (5H, H-5, H-9, H-18, H-19, H-20), 5.10 (1H, H-12), 5.38-5.47 (1H, H-3), 7.37-8.57 (5H, H-33, H-34, H-35, H-36, H-37) ppm; ^{13}C NMR (125, 500 MHz, DMSO) δ: 21.35 (C-1, C-2, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-14, C-15, C-16, C-17, C-18, C-19, C-20, C-21, C-22, C-23, C-25, C-26, C-27, C-28, C-29, C-30), 39.11-40.11 (-CH_3 of DMSO), 123.99-149.67 (C-33, C-34, C-35, C-36, C-37), 172.27 (C-11, C-24, C-31) ppm; Anal.

Calcd for $C_{32}H_{48}O_5$ (%): C, 88.9; H, 11.1; Found (%): C, 75; H, 9.4 (Figure 2).

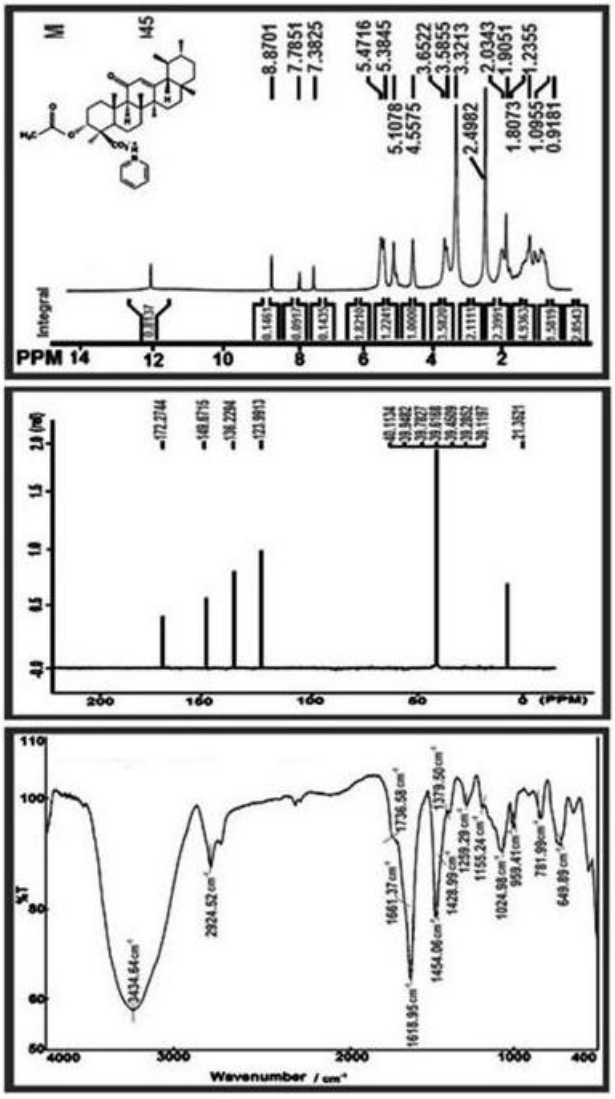


Figure 2: ^{1}H-NMR, ^{13}C-NMR and FT-IR spectra of AKBA (I).

Valine derivative of AKBA (KBA-Val) (II)

Yield, 31.4%; IR (KBr, cm^{-1}) υ: 1093.44 (-C-O-), Wide area of 1412.60 (-CH_3, -CH_2-), 1566.88 (-C=C-), 1658.48 (-COO^-, -NH-CO-), 1658.48 (-C=C-CO-, ring), 2932.23 (-CH-), 3426.89 (-NH-, -OH); MS $(M+1)^+$, m/z: 607 ($C_{35}H_{54}NO_5K^+$), 593 ($C_{34}H_{53}NO_5K^+$), 578 ($C_{33}H_{49}NO_5K^+$), 564 ($C_{32}H_{47}NO_5K^+$), 552 ($C_{35}H_{54}NO_4^+$), 538 ($C_{34}H_{52}NO_4^+$), 524 ($C_{34}H_{54}NO_3^+$), 510 ($C_{33}H_{52}NO_3^+$), 496 ($C_{32}H_{50}NO_3^+$), 482 ($C_{31}H_{48}NO_3^+$), 467 ($C_{30}H_{45}NO_3^+$), 451 ($C_{29}H_{41}NO_3^+$); Anal. Calcd for $C_{35}H_{54}NO_5K$ (%): C, 86.1; H, 11.1; N, 2.8; Found (%): C, 69.2; H, 8.9; N, 2.3 (Figure 3).

Alanine derivative of AKBA (KBA-Ala) (III)

Yield, 31.6%; IR (KBr, cm^{-1}) υ: 1020.16 (-C-O-), 1360.53 (-CH_3), 1451.17 (-CH_2-), 1569.77 (-C=C-), 1647.88 (-COO^-, -NH-CO-, -C=C-CO-), 2911.99 (-CH-), 3510.77 (-NH-, -OH); MS $(M+1)^+$, m/z: 579 ($C_{33}H_{50}NO_5K^+$), 523 ($C_{33}H_{49}NO_4^+$), 495 ($C_{32}H_{49}NO_3^+$), 451 ($C_{30}H_{43}O_3^+$), 423 ($C_{29}H_{43}O_2^+$), 393 ($C_{27}H_{37}O_2^+$), 363 ($C_{25}H_{31}O_2^+$), 335 ($C_{23}H_{27}O_2^+$), 334 ($C_{23}H_{26}O_2^+$), 313 ($C_{23}H_{21}O^+$), 285 ($C_{21}H_{17}O^+$); Anal. Calcd for $C_{33}H_{50}NO_5K$ (%): C, 86.1; H, 10.8; N, 3.1; Found (%): C, 68.4; H, 8.6; N, 2.4 (Figure 4).

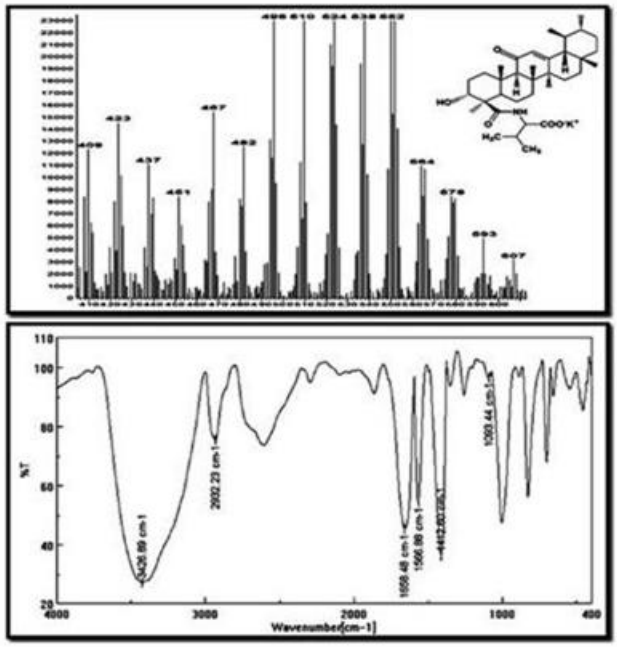


Figure 3: GC-MS and FT-IR spectra of compound (II). The presence of 1658.48 and 3426.89 cm^{-1}peaks in FT-IR spectrum confirmed the presence of amide, carbonyl, carboxyl and N-H, O-H functional groups, respectively. The peak at *m/z* 607 is due to the fragment $\{K[C_{35}H_{54}NO_5]\}^+$ which indicated the formation of compound II and other peaks that were detected by GC-mass confirmed the suggested mechanism.

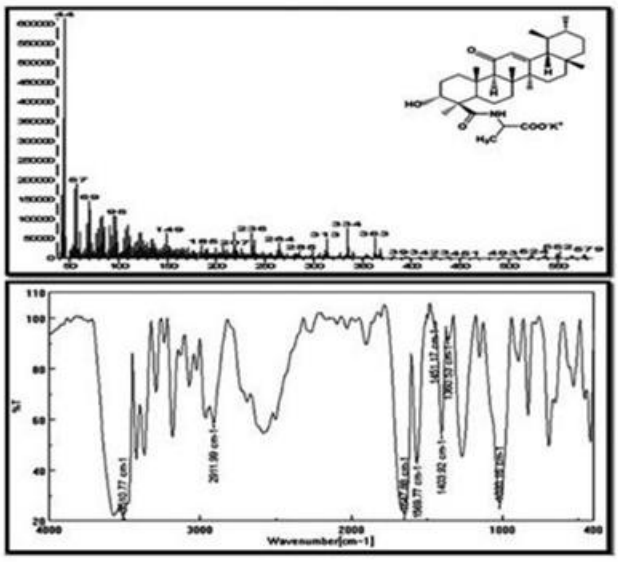


Figure 4: GC-MS and FT-IR spectra of compound (III). The presence of 1647.88 and 3510.77 cm^{-1}peaks in FT-IR spectrum confirmed the presence of amide, carbonyl, carboxyl and N-H, O-H functional groups, respectively. The peak at *m/z* 579 is due to the fragment $\{K[C_{33}H_{50}NO_5]\}^+$ which indicated the formation of compound III and other peaks that were detected by GC-mass confirmed the suggested mechanism.

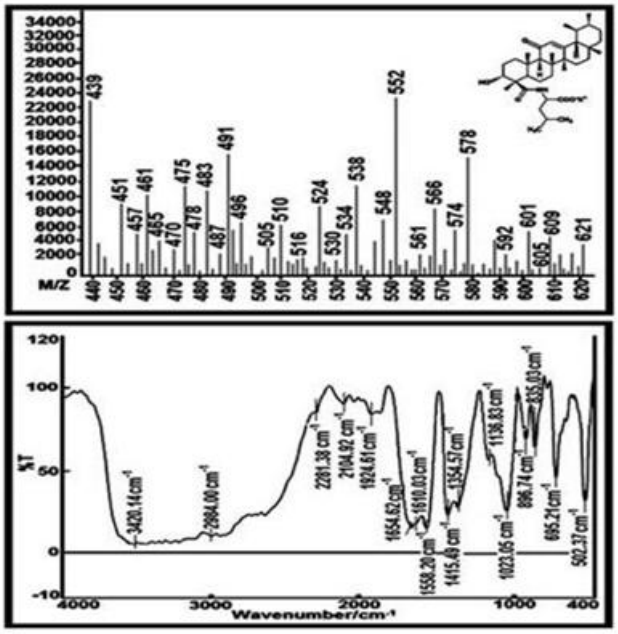


Figure 5: GC-MS and FT-IR spectra of compound (IV). The presence of 1654.62 and 1610.03 cm^{-1}peaks in FT-IR spectrum confirmed the presence of amide and carboxyl group and 3420.14 cm^{-1} peak is representative for formation of N-H and O-H functional groups that display leaving of acetate and pyridine groups. The peak at *m/z* 621 is due to the fragment $\{K[C_{36}H_{56}NO_5]\}^+$ which indicated the formation of compound IV and other peaks that were detected by GC-mass confirmed the suggested mechanism.

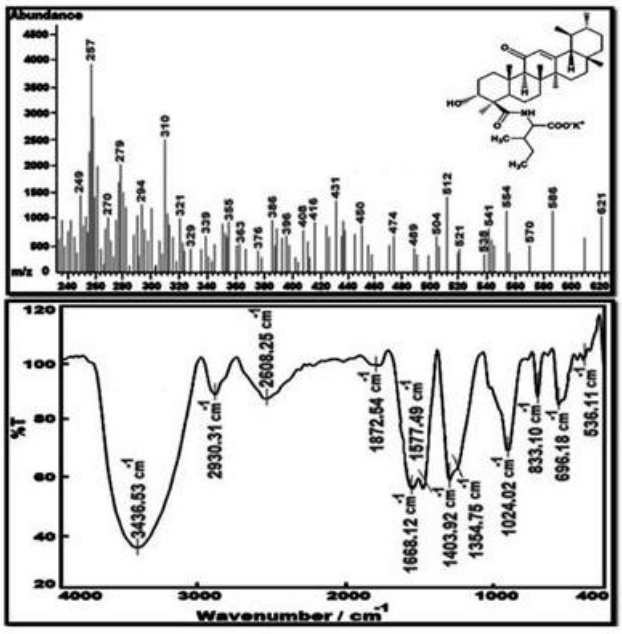


Figure 6: GC-MS and FT-IR spectra of compound (V). The presence of 3436.52 cm^{-1} peak in FT-IR spectrum confirmed the presence of amide and hydroxyl group that confirm leaving of acetate and pyridine groups. The peak at *m/z* 621 is due to the fragment $\{K[C_{36}H_{56}NO_5]\}^+$ which indicated the formation of compound V and other peaks that were detected by GC-mass confirmed the suggested mechanism.

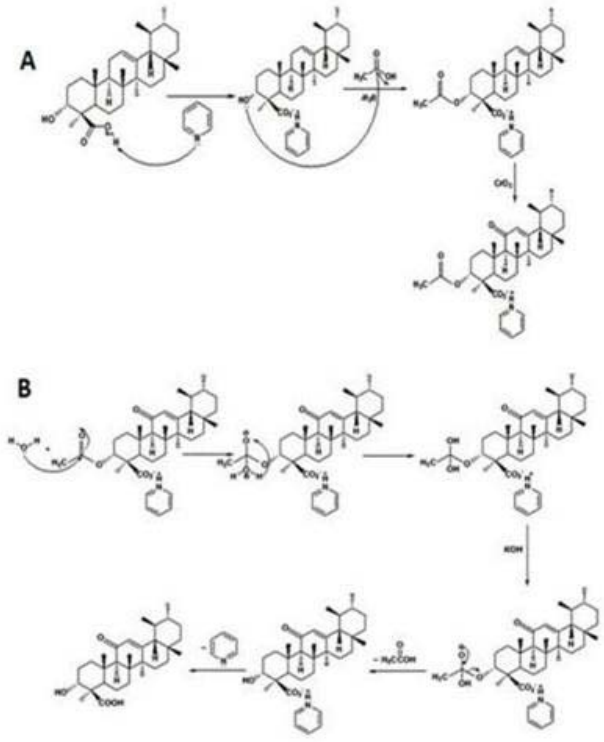


Figure 7: Mechanism of synthesis of AKBA (a) and KBA (b). (a) The formation of AKBA is initiated based on nucleophilic attack of pyridine to carboxyl group of BA and ultimately, C11 is oxidized by addition of chromium trioxide (CrO_3). (b) KBA is an intermediate compound that is formed in the coupling process between amino acids and AKBA.

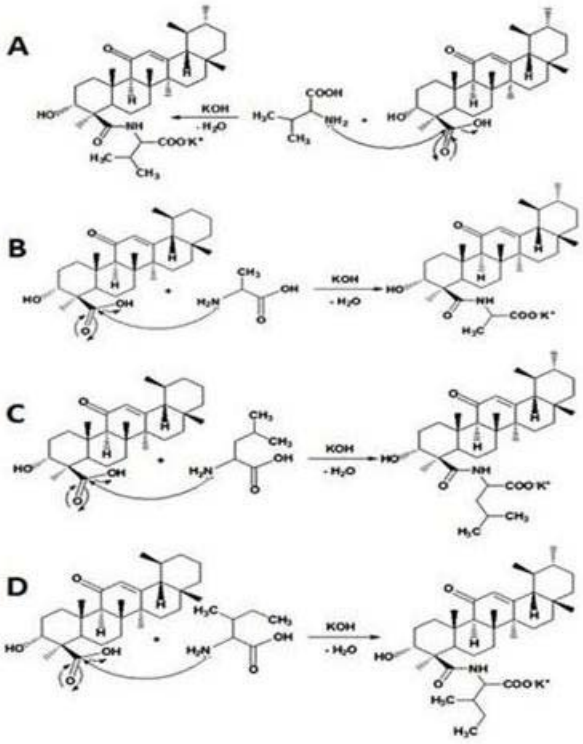


Figure 8: Mechanism of coupling reactions of valine (a), alanine (b), leucine (c) and isoleucine (d) with KBA. Compounds (II-V) are gained based on amidation reaction.

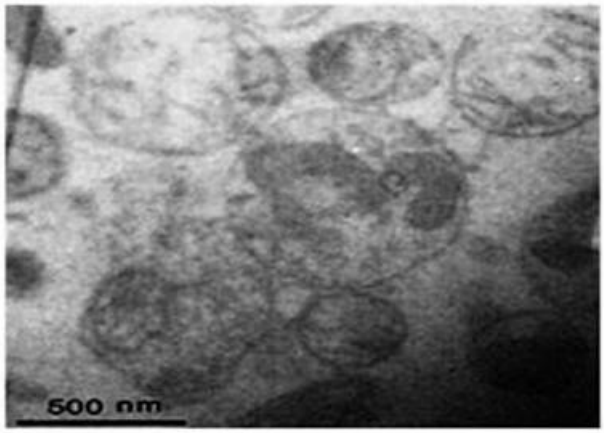


Figure 9: Electron microscopy image of sheep hemispheres synaptosomes displaying the normal morphology of synaptosomes.

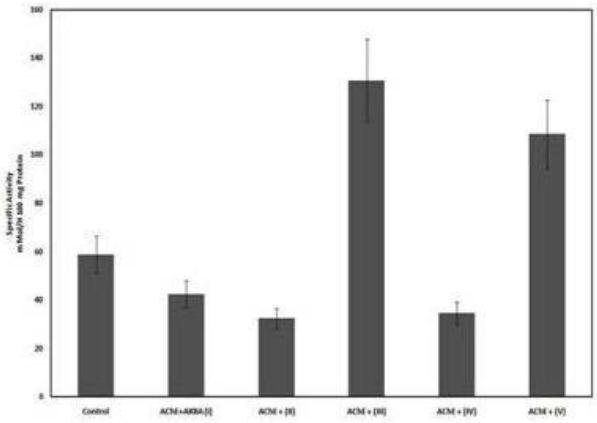


Figure 10: Effects of compounds (I-V) on AChE activity. Specific activity of AChE was measured after incubation of 1 mg/ml of synaptosome suspension with 0.1 mg of each synthesized compounds. The enzyme activity was assayed at 37°C and the absorbance was detected at 412 nm.

Leucine derivative of AKBA (KBA-Leu) (IV)

Yield, 31.7%; IR (KBr, cm^{-1}) υ: 1354.57 (-CH_3-), 1415.49 (-CH_2-), 1558.20 (-C=C-), 1654.62, 1610.03 (-NH-,-COO^-K^+, -CO-), 2984.01 (-CH-), 3420.14 (-NH, OH); MS $(M+1)^+$, m/z: 621 ($C_{36}H_{56}NO_5K^+$), 605 ($C_{36}H_{56}NO_4K^+$), 578 ($C_{33}H_{49}NO_5K^+$), 566 ($C_{36}H_{56}NO_4^+$), 552 ($C_{35}H_{54}NO_4^+$), 538 ($C_{34}H_{52}NO_4^+$) or ($C_{35}H_{56}NO_3^+$), 524 ($C_{33}H_{50}NO_4^+$), 510 ($C_{32}H_{48}NO_4^+$) or ($C_{33}H_{52}NO_3^+$), 496 ($C_{33}H_{52}O_3^+$), 478 ($C_{33}H_{50}O_2^+$), 465($C_{32}H_{49}O_2^+$); Anal. Calcd for $C_{33}H_{50}NO_5K$ (%): C, 86.06; H, 11.15; N, 2.78; Found (%): C, 70; H, 9.1; N, 2.3 (Figure 5).

Leucine derivative of AKBA (KBA-Ileu) (V)

Yield, 32.6%; IR (KBr, cm^{-1}) υ: 1024.02 (-C-O), 1354.75 (-CH_3-), 1403.2 (-CH_2-), 1577.49 (-C=C-), 1668.12 (-ring, salt, amide, C-O), 2930.31 (-CH-), 3436.52 (-NH, OH); MS $(M+1)^+$, m/z: 621 ($C_{36}H_{56}NO_5K^+$), 541 ($C_{35}H_{59}NO_3^+$), 538 ($C_{35}H_{56}NO_3^+$), 521 ($C_{35}H_{55}NO_2^+$), 512 ($C_{33}H_{54}NO_3^+$) or ($C_{33}H_{54}NO_3^+$), 504 ($C_{35}H_{54}NO^+$), 489 ($C_{34}H_{51}NO^+$), 474 ($C_{33}H_{48}NO^+$), 431 ($C_{30}H_{41}NO^+$), 416 ($C_{29}H_{38}NO^+$), 386 ($C_{27}H_{32}NO^+$); Anal. Calcd for $C_{33}H_{50}NO_5K$ (%): C, 86.06; H, 11.15; N, 2.78; Found (%): C, 70; H, 9.1; N, 2.3 (Figure 6).

Chemistry

The formation of 3-O-acetyl-11-keto-beta-boswellic acid (AKBA) is initiated based on nucleophilic attack of pyridine to carboxyl group of BA. Then, the OH group located on C3 of intermediate attacks to carbonyl group of added acetic acid and C11 is oxidized by addition of chromium trioxide (CrO_3) in order to yield AKBA (compound I) (Figure 7a). The presence of carbonyl and pyridine ring moiety was detected by broad absorption at 1661.37 and 3434.64 cm^{-1}, respectively in IR spectrum (Figure 2).

Compounds (II-V) are gained based on amidation reaction. First, the water molecule adds to carboxyl group of AKBA through nucleophilic nature of oxygen. Then, acetate and pyridine act as leaving groups and are eliminated from the compound in basic medium that is made by addition of KOH in order to constitute 11-Keto-β-boswellic acid (KBA) (Figure 7b). Finally, the amine group of amino acids (valine, alanine, leucine and isoleucine), which acts as a nucleophile, attacks to the carboxyl group located on C4 due to the unpaired electron of nitrogen and consequently, amide compounds are formed (compounds II, III, IV and V, respectively) (Figure 8).

Inhibitory effects on AChE activity

Synaptosomal AChE specific activity was 58.74 mmol /h 100 mg protein ± 4.70 as control enzyme activity. In addition, normal morphology of synaptosomes was observed by TEM (Figure 9). The impact of new compounds (I-IV) on the AChE activity was investigated in vitro in order to evaluate whether such agents can be exploited in the treatment of AD or not. As expected, a whit inhibitory effect of AKBA was displayed as decreased AChE activity by 27% (Figure 10). On the other hand, conjugation of AKBA with two amino acids increased its inhibitory efficacy further. We showed that the inhibitory performance of AKBA was significantly improved when combined with valine and leucine that caused AChE decrease 45% and 41%, respectively compared to control. It seems that the inhibitory effects of aforementioned conjugates of AKBA are similar. Although compounds II and IV decreased AChE activity, compounds III and V elevated incredibly the AChE activity in comparison with control, 123% and 85% respectively (Figure 10).

DISCUSSION

AD is a neurodegenerative disorder recognized by its progressive nature of the degeneration of the hippocampal and cortical regions that causes impairment of memory and cognitive ability. It is one of the most common causes of clinical dementia in elderly people. Many factors are related to increased risk for developing AD later in life.[21] The studies have shown that the incidence of AD doubles every 5 year between 65 and 85

years in every human population investigated.[22] According to the international world Alzheimer report published in 2010, about 35.6 million individuals were living with symptoms of the disease like dementia worldwide which this number would raise to 65.7 million persons by 2030 and 115.4 million people might probably have the disease by 2050.[23] Such people, their family, relatives and friends suffer at personal, social and financial level.

The genesis of reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) at moderate concentration leads to natural physiological actions. But the excess concentrations of ROS and RNS or its inefficient removal and elimination by body's antioxidant system leading to possible biological impairment is called oxidative stress and nitrosative stress.[24] It is believed that oxidative stress is the one of major causative elements in generation of many degenerative and chronic diseases like AD.[25,26] Moreover, inflammation of the brain is another factor in the pathogenesis of AD. In the end, as mentioned earlier, deficiency of ACh is a critical factor in the generation of the symptology of AD.[27] It has been proposed that cholinergic agents, either AChE inhibitors or cholinergic agonists might be effective in improving clinical symptoms of AD.[28] The AChE active site contains a narrow gorge with two discrete binding sites for ligands; hydrolysis occurs at the bottom of the gorge which is called A-site (an acylation site) and a P-site (a peripheral site) at the gorge mouth.[29] AChE inhibitors can be applied as a treatment option for human diseases or more infamously as chemical warfare agents and weapons.

Plants are the extremely valuable source of novel pharmaceutical agent developments. The aim of the current study is to evaluate the AChE inhibitory activity of salts of the valine, alanine, leucine and isoleucine amino acid derivatives of AKBA (II, III, IV, and V) and to compare it with AKBA (I) alone. The only agents that have been approved by the Food and Drug Administration (FDA) for the treatment of AD are AChE inhibitors. All other kinds of pharmaceutical drugs have not been approved and are administered on an off-label basis.[30] As illustrated in Figure 10, out of four compounds investigated for their AChE inhibitory activity, compounds II and IV was found to be more potent compared to AKBA as the standard inhibitor (specific activity 32.37, 34.54 for compounds II and IV, respectively compared to 42.7 for AKBA). On the other hand, the studies showed that combination of AKBA with alanine and isoleucine reduced the inhibitory effect of AKBA on AChE specific activity (specific activity 130.86, 108.6 for compounds III and V, respectively compared to 42.7 for AKBA). It seems that AKBA in combination with valine and leucine has the strongest activity against AChE among the synthesized compounds and apparently they can bind and block the active site of the enzyme. The results of the present study reveal that particular amino acid compounds of Boswellia have more protective and therapeutic efficiency compared to Boswellia alone.

Yassin et al.[30] have investigated the effects of aqueous infusions of B. Serrata for treatment of AD induced by $AlCl_3$ in rats. They reported that animals' treatment with Boswellia increased their activity significantly and examination of the brain tissue of those rats showed healthy neurons and that amyloid plaques had removed. In addition, a significant increase in the Ach levels as well as a significant decrease in the brain AChE levels in a dose dependent manner was reported when compared to the AD induced groups. It has been revealed that triterpene acids such as BA and AKBA show strong antioxidant capability and anti-oxidant activity and they concluded that compounds available in B. Serrata are beneficial and helpful in AD-induced rats.

A study done by Singh et al.[3] on the synergistic effect of boswellic acid mixture (BA) and glucosamine for anti-inflammatory and anti-arthritic activities in rats revealed that the combination shows anti0arthritic activity to a greater extent. In another study, a methanolic extract of Boswelliasocotrana has been discovered to have efficient anti-cholinesterase activities with 22.32% inhibition at 0.05 mg/ml while 0.2 mg/ml caused 71.21% inhibition and another investigated species (elongate) inhibited 11.23% and 46.34%, respectively.[31]

Many studies have been done on pharmacological activities of natural triterpenoids and their therapeutic implications. The studies also suggested molecular mechanisms regarding to its anti-inflammatory and anti-oxidant activity.[32,33] Our study revealed that some amino acid compounds of AKBA have more potent AChE inhibiting properties and hence resemble in the mode of action of drugs used for treatment of AD. Nevertheless, the exact mechanism by which these compounds interact with AChE, remains unexplored. Undoubtedly, molecular docking and simulation studies are required as a part of documentation process to improve the reliability and accuracy of our results and to investigate possible interactions among molecules.

CONCLUSION

This work strongly supports the idea of using AKBA, as a derivative of natural BA, combined with amino acids as a more potent means to improve pathogenesis of AD by decreasing AChE activity and as a result, promotion of ACh levels effective at inhibiting the progression process of AD. We showed that the coupling of AKBA with valine and leucine amino acids can successfully strengthen the effect of AKBA on decline of the AChE activity. The spectroscopic techniques were used to exactly confirm the coupling process in this study. Following the development optimized formulations, we characterized their efficiency at decreasing AChE activity compared to standard AKBA. The data obtained suggest that compound II and IV could be a potential promising and more potent alternative for available agents applied to treat AD.

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