Research Article

Synthesis of New Chamazulene Derivatives and the Study of Their Effects on Tubulin Polymerization

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ABSTRACT

The antiphlogistic action of Chamazulene has been verified and asserted in a variety of experimental inflammatory reaction. Also it has anti-inflammatory properties *in vivo*. In this study, we have performed the reaction of chamazulene with two different cyclic ketons. So, reaction between Chamazulene and Dimedone in an acidic condition has been accomplished. The ensued solution has been cooled, washed with water and dried over suitable absorbent. Experimental data about *in vitro* study showed that the new product was able to increase tubulin polymerization. On the other hand, Chamazulene and 3-methoxy-2-cyclohexene-1-one reacted together with similar method. The molecular structures of products were specified and confirmed by Analytical and spectroscopic methods (IR, ^{1}H-NMR).

Keywords: Chamazulene, Dimedone, Tubulin.

INTRODUCTION

Chamomile is used worldwide as a medicinal plant and it is one of the most important crops for pharmaceutical and cosmetic purposes in the world.[1,2] Chamazulene (CHA) that has been found in various types of plants such as chamomile (Matricaria Chamomilla), is an aromatic compound which is a degradation product of Chamazulene carboxylic acid as an artifact Matricine by distillation. Chamomile is commonly used to treat inflammation and spasms. Extracts obtained by different methods and major constituents isolated from the Chamomile have been tested successfully for anti inflammatory, anti spasmodic, sedative, antimicrobial, anti ulcerogenic and wound healing effects.[3] Chamazulene which is a blue violet derivative of Azulene, is also an aromatic chemical compound of Azulene parents that is biosynthesized from the sesquiterpene Matricine.

On the other hand, one of the several members of small family of globular protein is tubulin. Recent data have revealed that the tubulin super-family of proteins is much larger than was thought previously. Six distinct families within the tubulin super-family have been discovered and more might await discovery. Alpha-, beta- and gamma-tubulins are ubiquitous in eukaryotes. Alpha- and beta-tubulins are the major components of microtubules, and gamma-tubulin plays an important role in the nucleation of microtubule assembly.[4-7]

The most common members of the tubulin family are alpha-tubulin and beta-tubulin, the proteins that make up microtubules.[8] Tubulin is part of the "skeleton" of brain cells so to speak and this disappears or is greatly reduced in the Alzheimer's brain. Alzheimer's disease (AD) is a lentamente, not healable disease, special for human brain which so far remains unknown origin. Because the origin of AD is undefined, it is difficult to search for diagnostic tools or for a medication.[9]

One of the notable changes in an Alzheimer's brain is the loss of beta-tubulin. Since reduction of tubulin is a characteristic of Alzheimer's it might be worthwhile to synthesize new compounds with potential ability to increase tubulin polymerization.

MATERIALS AND METHODS

Instruments

The ^{1}H-NMR data were recorded with a Bruker 300 Avance Spectrometer in $CDCl_3$. IR spectra were obtained with a Jasco FT-IR -410 spectrophotometer by KBr pellet method.

We used UV Carry 100 Bio Varrian instrument to generate absorbance spectra.

Materials

All reagents used were purchased from Merck Company. All chemicals were reagent grade and were without further purification.

Preparation of 1-(3-acetoxy-5,5-dimethylcyclohex-2-en-1-ylidene)-5-ethyl-3,8-dimethyl-1,4-dihydroazulen-4-ylium perchlorate (1)

A mixture of Chamazulene (0.37 g) and Dimedone (0.2 g) was heated at 70 °C for 7 min. After that acetic acid (1 ml) was added to the reaction mixture under reflux condition. In continue Perchloric acid (0.25 ml) was added and reflux process was continued for more 5 minutes.

After mixture cooling and dilution with ether, the organic layer was washed with water and sodium hydrogen carbonate solution, then dried on Na_2SO_4 and evaporated.

Preparation of 5-ethyl-3,8-dimethyl-1-(3-oxocyclohexylidene)-1,4-dihydro azulen-4-ylium (2)

In a similar method, compound (2) was synthesized by the reaction of Chamazulene (0.37 g.), and 3-methoxy-2-cyclohexene-1-one (0.28 g) under reflux condition.

Animal

Twenty laboratory mice with average brain weight 190 to 200 g were anesthetized with chloroform. After the mice heads were cut, their brain removed and washed several times with cold physiologic serum NaCl (normal saline). Protein extraction was performed using buffer PEM (Pipes -0.4M, EGTA 20mμ, $MgCl_2$ 20 mμ) followed by blending (blender: Panasonic M1225G). Brain tissue was homogenized in buffer PEM, using a tissue homogenizer (Stayer TM531).

After centrifugation for 50 minutes at 16000 rpm (Sigma-3-30K UK), PEM was added to the supernatant at 4° C. Then Mg-GTP (Guanosine-5'-triphosphate) was added and the mixture was incubated for 45 minutes to put up a complete tubulin polymerization.

After centrifugation for 45 minutes at 35° C (35000 rpm, Beckman 25-50 USA) the mixture was cooled up to 4°C and PEM was added to microtubule filament-containing sediment. The product of mixing was homogenized for 30 minutes and centrifuged for 40 min at 4°C.

The above process was repeated to complete the polymerization and the supernatant was collected and stored in the liquid nitrogen in -70° C, until using.

RESULTS

FT-IR and ^{1}H-NMR spectra

Both of synthesized compound (1) and (2) were characterized by FT-IR and ^{1}H-NMR spectroscopy. Infrared spectroscopy is an appropriate technique in order to functional groups identification in a molecule which can lead to qualitative analysis of different materials.

^{1}H-NMR is a preeminent technique for determining the structure of organic compounds. Of all the spectroscopic methods, it is the only one for which a complete analysis and interpretation of the entire spectrum is normally expected. Hence FT-IR and 1H-NMR spectroscopy were utilized to evaluate the structure of synthesized compounds (1) and (2) that were indicated below (figures 1,2, 1a-c, 2a-c).

Spectroscopic data of compound (1)

FT-IR (KBr): ῡ=Cm^{-1}: 3023.84 (CH), 2938.98 (CH), 1727.91 (C=O), 1434.28 (CH_2), 1372.20 (CH_3), 1214.93 (C-O), 759.82 ($ClO4^-$)

^{1}H-NMR (300MHz): $CDCl_4$, δ= ppm: (4H (Aromatic),7. 18-7.58), (H (HC=C), 5-5.7), (H (CH_2), 4-4.5), (H (CH_2), 3.6 - 4.1), (2H (CH_2), 3.5, 3.9-4), (3H (CH_3-C=O), 2.3-2.2), (H (CH_2), 2-2.1), (15H (5CH_3), H (CH_2), 0-1.5)

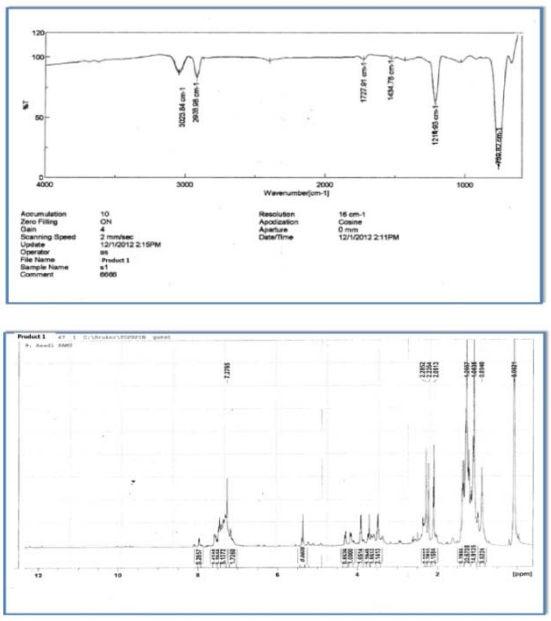


Figure 1: FT-IR and ^{1}H-NMR spectra of compound (1). The ratio of integrated data presented at ^{1}H-NMR spectrum affirmed two diastereomeric forms of compound (1).

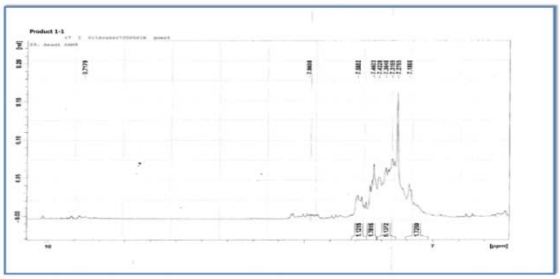


Figure 1a: Expanded ^{1}H-NMR spectrum of compound (1) part (a).

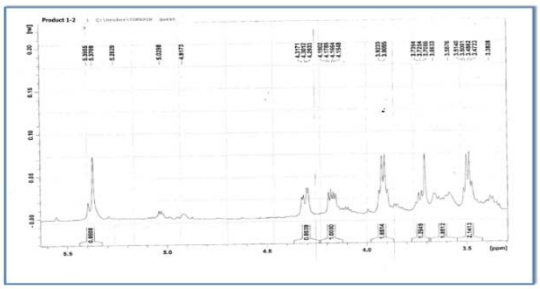


Figure 1b: Expanded ^{1}H-NMR spectrum of compound (1) part (b).

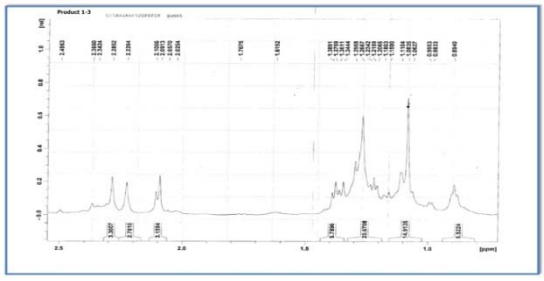


Figure 1c: Expanded ^{1}H-NMR spectrum of compound (1) part (c).

Spectroscopic data of compound (2)

FT-IR (KBr): ῡ=Cm^{-1}: 3023.84 (CH), 2938.98 (CH), 1727.19 (C=O), 1531.20 (C=C),1434.78 (CH_2),(759.82 (ClO_4^-)

^{1}H-NMR (300MHz): $CDCl_4$, δ= ppm: (4H (Aromatic), 7.06-7.24, 5.2-5.3), (2H (CH_2), 4.14-4.31), (H (CH_2), 3.67-3.83), (6H ($2CH_3$), 2.2-2.3), (3H (CH_3), 2H (CH_2), 2-2.1), (4H ($2CH_2$), H (CH_2), 1.2-1.37)

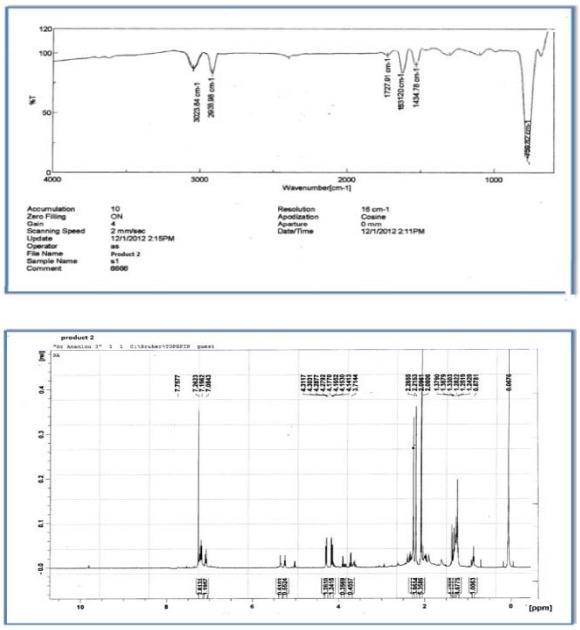


Figure 2: FT-IR and ^{1}H-NMR spectra of compound (2). The ratio of integrated data presented at ^{1}H-NMR spectrum affirmed two diastereomeric forms of compound (2).

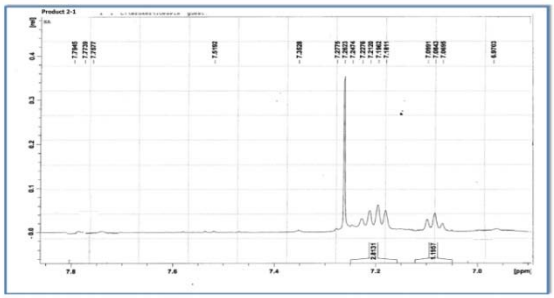


Figure 2a: Expanded ^{1}H-NMR spectrum of compound (2) part (a).

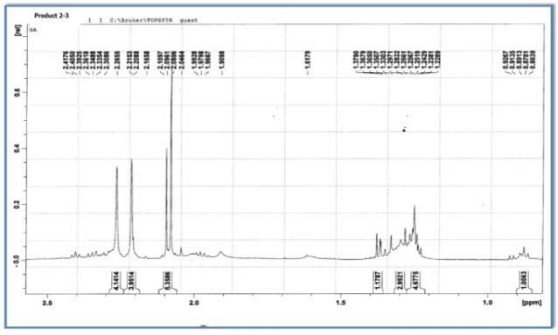


Figure 2b: Expanded ^{1}H-NMR spectrum of compound (2) part (b).

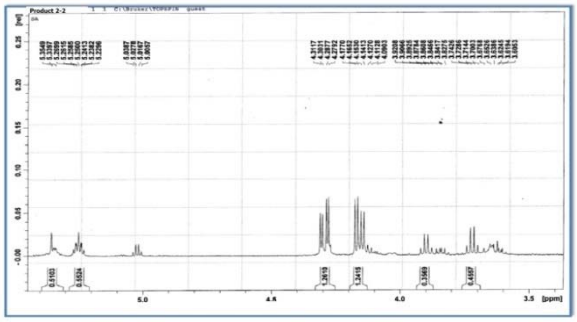


Figure 2c: Expanded ^{1}H-NMR spectrum of compound (2) part (c).

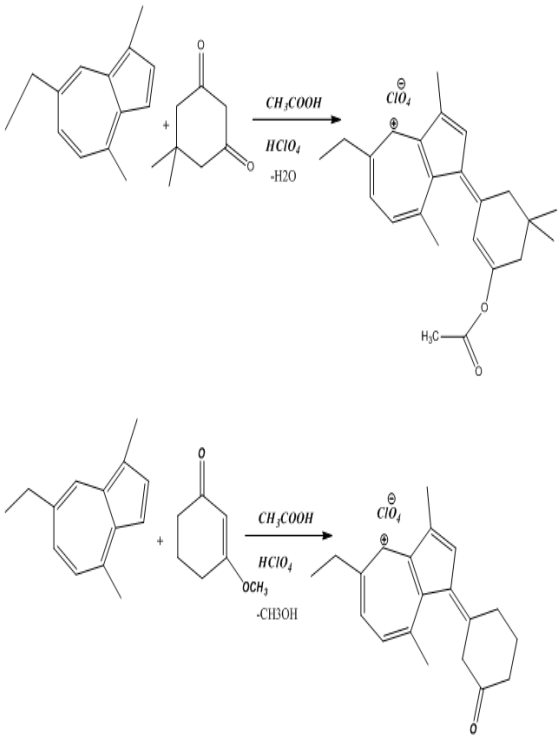
Tubulin Polymerization

The obtained materials were mixed with extracted microtubules of mouse brain and the UV-spectrums were taken at a wavelength of 350 nm by using CARY 100 CONC-Spectrophotometer-UV-VIS. The results were displayed as the absorbance graph against time for polymerization of tubulins. Experimental data showed both of these new compounds were able to increase the speed and amount of tubulin polymerization on mouse brine.

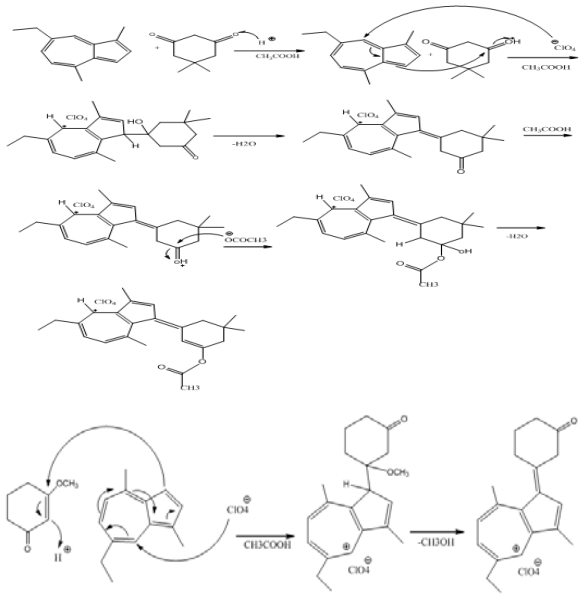
DISCUSSION

A primary insignia of Alzheimer disease is the environmental disorientation which leaves increased load on the caretakers for monitoring continuation. Investigations have proposed that poor spatial memory and navigational/spatial abilities are associated with low level of Testosterone in both humans and animals.[10-15] Compound which are polycyclic and have double bond in their molecular structure, such as frankincense, increase memory. Among the similar compounds in our body, we may refer to testosterone which increases IQ when high level in the brain and decreases IQ when low level in the brain and cause idiocy. Also on the other hand Adenosine Tri Phosphate (ATP), Guanosine-5'-triphosphate (GTP), Boswelic acid, Estrogen and Testosterone are such martial that have an impact and affect on memory. Moreover, the low levels of testosterone are associated with increased risk for developing in AD over the time and absolute free of health status, age, or education. Meanwhile in confronting to controls , and patients with Parkinson disease and who that are associated with high plasma amyloid beta peptide 1–40 levels, the levels of Testosterone remain low with the onset of AD.[16] The fast axonal transport is essential for the renovation of axons and membranes in the nerve terminal . Microtubules are the stem organelle for performing this transport. Therefore, assembly and stabilization of defective microtubule in neurons could lead to impaired axonal transport and abnormal synaptic transmission. There are a number of ways to stable the microtubules in neurons. These are including tubulin posttranslational modification and the regulation of microtubule associated proteins (MAPS) of either the high-molecular-weight MAPS or the low molecular weight tau proteins.[17] The main building

block of microtubules from AD brain ,the tubulins , are functionally proper for reassembling and forming the microtubules in vitro, but microtubule assembly from AD brain homogenates is not apperceived.[18] In this study, Chamomile was considered which contains Chamazulene. To work on living cells, we would need to find the similar material as same as the material in vivo. So Chamazulene was selected because of its structural similarity to Antihistamines and for its aromatic nature, pharmacological similarities to Antihistamines as well as Antioxidant, and having anti-inflammatory properties. Here, we studied the effect of Chamazulene and its two new synthesized derivatives on tubulin activities. Dimedone (5, 5-dimethyl-cyclohexane,1,3-dione) and 3-methoxy,2-cyclohexn-1-one were reacted with Chamazulene in two individual reaction. Although no reaction between Chamazulene and Dimedone was conducted in alkaline medium and in the presence of methanol, a yellow viscous mixture of 1-(3-acetoxy-5,5-dimethylcyclohex-2-en-1-ylidene)-5-ethyl-3,8-dimethyl-1,4-dihydroazulen-4-ylium perchlorate was obtained in acidic reaction condition. Because of non planar molecular structure of Chamazulene, the product includes two diastereomeric forms with ratio of 50-50% which was estimated of integrated data presented at ^{1}H-NMR spectrum. In the same process, reaction between 3-methoxy,2-cyclohex-1-on and Chamazulene lead to production of compound 5-ethyl-3,8-dimethyl-1-(3-oxocyclohexylidene)-1,4-dihydroazulen-4-ylium perchlorate which was formed as diastereomeric pairs with ratio of 38.5-61.5 %. Suggested synthetic reaction pathways and mechanisms for preparation of compounds (1) and (2) are illustrated in schemes 1 and 2.



Scheme 1: Synthetic reaction pathways for preparation of compounds (1) and (2)



Scheme 2: Suggested reaction mechanism for preparation of compound (1) and (2)

Tubulin protein extracted from mouse brain was subjected to investigate the effects of new compound on speed and amount of tubulin polymerization. Tubulin protein (100 micro liter) and compound (1) (20 micro liter) were dissolved in DMSO and the mixture was fixed to 200 ml by adding of a solution of Guanosine-5'-triphosphate (GTP)(4 micro lit) prepared in PEM(pipes - 0.4M,egta 20mμ,$MgCl_2$ 20 mμ) buffer (pH=6.9). The polymerization activity of (1) was assayed at 37° C, and quantified by measuring absorbance at 350 nm using an ELISA micro-plate reader. Experimental data were calibrated to the appropriate calibration curve according to the time spent. The effect of compound (1) on tubulin polymerization was evaluated (table 1 and figure 3). The process was repeated for compound (2) (table 2 and figure 4) as well as Chamazulene (table 3 and figure 5).

Table 1. Symbol details in figure 1

1	B_2	Product (1) 20 µlit
2	B_4	Chamazulen 20 µlit
3	C_2	Product (1) 20 µlit repeat
4	C_4	Chamazulen 20 µlit repeat
5	C_8	Normal (control solution)

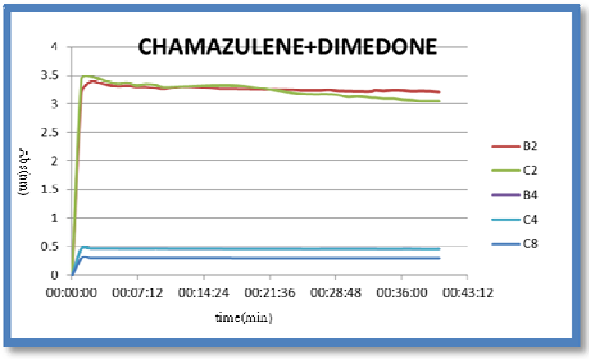


Figure 3: The effect of compound (1) on tubulin polymerization

Table 2: Symbol details in figure 2

1	B_3	Product (2) 20 µlit
2	B_4	Chamazulen 20 µlit
3	C_3	Product (2) 20 µlit repeat
4	C_4	Chamazulen 20 µlit repeat
5	C_8	Normal (control solution)

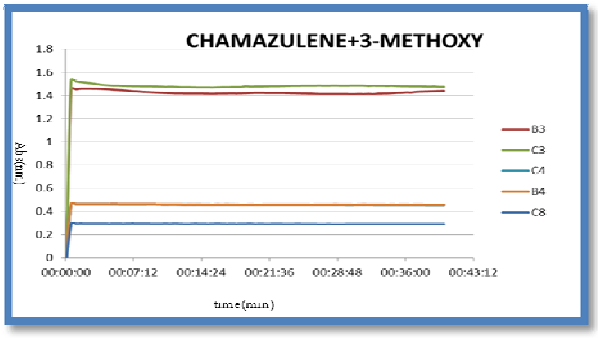


Figure 4: The effect of compound (2) on tubulin polymerization

Table 3. Symbol details in figure 3

1	B_4	Chamazulen 20 µlit
2	B_5	Chamazulen 50 µlit
3	C_4	Chamazulen 20 µlit repeat
4	C_5	Chamazulen 50 µlit repeat
5	C_8	Normal (control solution)

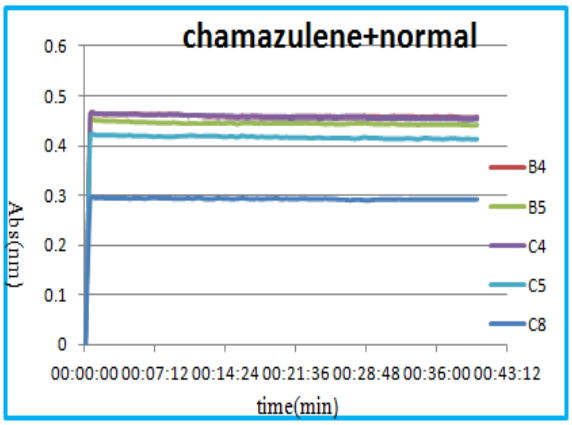


Figure 5: The effect of Chamazulen on tubulin polymerization

Based upon experimental data, the following results were obtained:

1) Considering data and graph, we noted that extracted Chamazulene effected, regardless of the concentration, on tubulin polymerization and increased the rate and value of polymerization of tubulins. Hence this compound is a proper material for tubulins polymerization improvement and changing them to microtubules.

2) With respect to the structure of the compound (1), we consider it has two parts. One part is hydrophilic and another is hydrophobic. This compound sits somewhere near Tue protein in tubulin occupies two Cysteine in beta area. Also it may sit in alpha area which such activity in this area can be considered negligible. Compound (2) can act as a ligand because of its ionic properties. It can affect the tubulin activity and cause aggregation. This may close dimmers together and make an amorphous set .This indicates the synthesized material increased the rate and value of polymerization which can be suitable for applying in Cancer and Alzheimer since it makes a rapid withdrawal of dimmers out of system.

CONCLUSION

Synthesize of a new Dimedone derivative has been successfully carried and the new product was capable to promote tubulin polymerization. Also synthesis of 3-methoxy-2-cyclohexene-1-one derivative was achieved with similar method.

According to the done research, we have found that the two synthesized substances along with pure Chamazulene, all three have the optimal effect on the mouse brain and increase the speed and amount of the tubulin polymerization. This is an important help to know that we can affect on production of microtubule by using these synthesized materials and applied effects on tubulin polymerization.

Considering that these microtubules are one of the important factors in Alzheimer with an impact on the amount of their production we hope that these new synthesized compounds could play an important role in designing effective precursors which control the Alzheimer Disease.

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