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Synthesis And Characterization, Some Azide Compounds Loaded on The Liposomes and Studying Their Effectiveness as Antioxidants and Cancer

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Abstract

The study included the preparation of three types of chalcons compound (T1, T2, and T3) through the interaction of addition (4-azidophenylethanone) with some types of aldehydes (oxochromane-3-carbldehyde , 4-dimethyl ameno benzaldehyde,3-nitro benzaldehyde) With the presence of a basic medium, the prepared compounds were diagnosed with FTIR infrared technology, 1H-NMR technology, 13C-NMR, and Mass-spectra technique. The effectiveness of these compounds against oxidative stress was studied on the cell line HCAM and good results were shown. The prepared azide compounds were loaded on liposomes which were prepared from lecithin. The effect of the acidic function on the liberation of the prepared compounds from the layers of lipid nanoparticles was also studied at the functions (pH = 7.2), stomach fluid (pH = 1.2) and intestines (pH = 8.2) and using ultraviolet spectroscopy at (37°C). The effectiveness of the liposomes loaded against human liver cancer HCAM and the rate of inhibition at a concentration of 1000 micrograms/mol were measured and the lethal half concentration IC50 was calculated using different concentrations (10,100,200,500,750) micrograms/mol using the DMSO as solvent, They showed good results and high efficacy. The comparison was also made using a TEM electron microscope of the liposomes in the presence and absence of the prepared compounds.

Introduction

Azide is an important functional group in organic chemistry because it is used in the synthesis of most organic compounds, N3 is the active group in it;[1] Among its main sources are amines;[2]as it is considered the most widespread functional group in organic chemistry, as well as its commonness in natural and pharmaceutical products that are distinguished by heterogeneous atoms. It has been proven that some derivatives of azide are strong anti-tumor agents and cancer, as in the examples shown below for some azide derivatives as anti-cancer agents;[3] The

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azide group is characterized by the physical and chemical properties of this group's polarity ;[4,5].

The Chalchone are aromatic, ketones α, βwith unsaturated carbonyl compounds, also known as diphenylketonuria and styrenephenylketonuria, formed as a result of condensation between acetophenone and benzaldehyde under alkaline conditions;[6]. These compounds possess various biological and pharmacological efficacy due to their structure, possessing anti-inflammatory, antimicrobial, anti-tumor, antioxidant, antiviral, and other efficacy;[7,8]. The researcher Ananda M. Arasavelli and his group [9] were able to prepare some galcon derivatives with anti-cancer properties, as in the following equation:

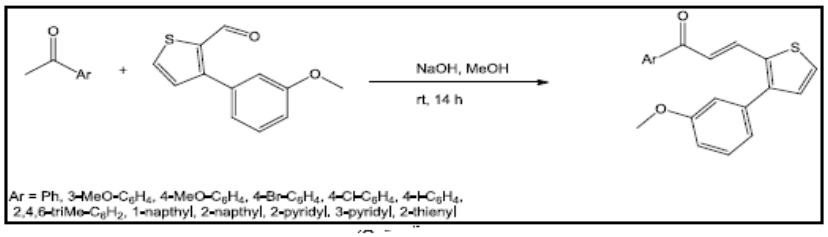


Figure 1: Galcon derivatives that have anti-cancer activity

Liposomes are two-layer globular vesicles based on lipids. In 1965, it was discovered and described for the first time as a puffy phospholipid [10]. A few years later, the structural composition of liposomes was discovered by Bangham et.al. Small devices made of two or more bilayer phospholipids are enclosed. Due to the variety of particle sizes, from 20 nm to several micrometers. Lipid vesicles are either nanoparticles or fine particles that have the ability to encapsulate materials. The different and polar nature [11,12] as well as the liposomes showed great importance as compounds. It is biologically active and is involved in the pharmaceutical and cosmetic industries. These applications have evolved to include the food and agricultural industries, as they have been used as antioxidants and cancer treatments because of their biocompatibility, biodegradability, and low toxicity. The ability to encapsulate a variety of drugs makes liposomes attractive. As carriers of highly therapeutic drugs, target therapeutic drugs and imaging agents are used. Carriers of nanoparticles and their delivery have made great progress [13]. Liposomes are made of non-toxic cholesterol and phospholipids. Particles are formed from a two-layer (single-layer) or multiple-layer (multi-layer) lipid surrounding one layer of water. The polar and hydrophilic head groups are directed towards the aqueous phase as they are directed. Non-polar hydrophobic tail groups away from water ;[14] Figure (1,2).

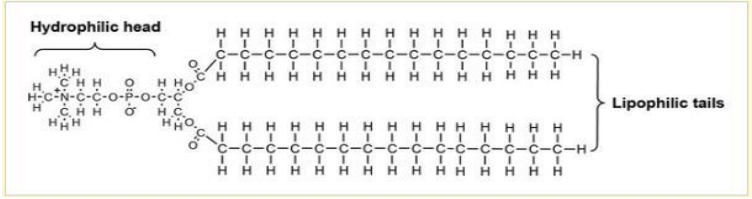


Figure 2: The chemical structure of the phospholipid molecule

Experimental Section

Double-distilled water and chemicals of the highest purity used in all experiments were supplied by Fluka and Merck. Melting points were determined on a thermal scientific apparatus and are uncorrected. IR spectra were recorded on a FT-IR spectrophotometer using KBr (ν cm-1). 1H-NMR and 13C-NMR spectra were recorded on a Bruker (400 MHz) spectrometer in Iran, using DMSO and CDCl3 as solvents and TMS as an internal standard, with chemical shifts reported in ppm (s). Mass spectra were obtained on an Agilent Technology (HP) model 5973 spectrometer in Iran, at Tehran University.

The purity of products and progress of reactions were checked by TLC on silica gel plates.

Preparation of Chalcone Compounds

(General method)

The compounds were prepared by dissolving equimolar amounts (0.002 mol) of aldehyde derivatives (4-nitrobenzaldehyde, 4-oxochromane-3-carbaldehyde, 4-dimethylaminobenzaldehyde) in 10 mL of chloroform in a beaker. The prepared azide (1-(4-azidophenyl)ethanone) was dissolved in 10 mL of chloroform and added to the first solution. Then, 5 mL of 10% sodium hydroxide solution was added gradually to the mixture until the solution turned yellow, and after 4 hours of continuous stirring, a precipitate formed. The mixture was then acidified with hydrochloric acid. The reaction was monitored by TLC using a purification solvent (ethanol:chloroform, 2:8). The reaction mixture was left at room temperature, and the resulting precipitate was filtered, dried, and recrystallized from absolute ethanol. Table 1 shows the six different α,β-unsaturated ketones (chalcones) prepared using the Claisen-Schmidt method in basic media.[14]

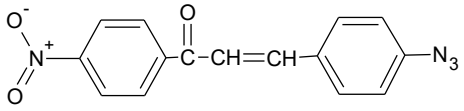
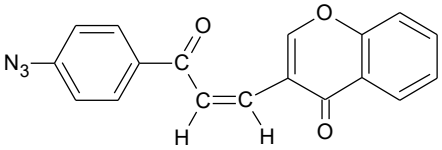
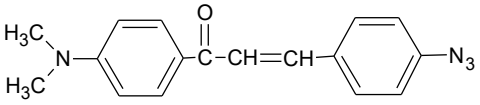
code	Structure	M.p C°	Yield %	Color / State
T_1		158	88%	White powder
T_2		124	87%	Yellow powder
T_3		91	75%	Yellow powder

Table 1: The Prepared Chalcones , Physical data, and their structure

Preparation of liposome

The liposome was prepared using a thin film method. This method involves making a thin lipid film in a round-bottom flask by the removal of the organic solvent. One hundred mg of Soya lecithin and 30 mg cholesterol were dissolved in an equal 1: 1 mixture of chloroform and methanol in the 50 mL round-bottom flask. After the mixing process was complete, the solvent was then completely removed under vacuum using a rotary evaporator at (45-60) °C. The dried lipid film was hydrated with (10ml) of phosphate buffer solution (pH 7.2), which contains 3 mg of the prepared organic compound (T1, T2, and T3). The dispersion was left undisturbed at room temperature for 24 hours to allow complete swelling of the lipid film and hence to obtain vesicular [15]

Results And Discussion

The chemical structure of the synthesized compounds was characterized by using TLC and FT.IR ,1H, 13C-NMR spectroscopy, and mass spectroscopy.

The IR spectrum shows the prepared compounds that characterized by the appearance of the carbonyl group in the range from cm-1 1708-1649 and also the appearance of the characteristic C = C beam in the range from cm-1 1598-1541 as in the figure (3,4,5). All the 1HNMR spectra of the prepared compounds were also characterized by the emergence of double-beam signaling of the galactic double-block protons at the range 7-8As in the figure (6,7,8). The 13CNMR spectrum was characterized by the appearance of two C = C beams at the site 120ppm and a C = O beam at 190ppmAs in the figure (9,10,11). The mass spectrum was also characterized by the appearance of molecular ion peaks and the relative ion of the prepared compounds in the figure (12.13.14).

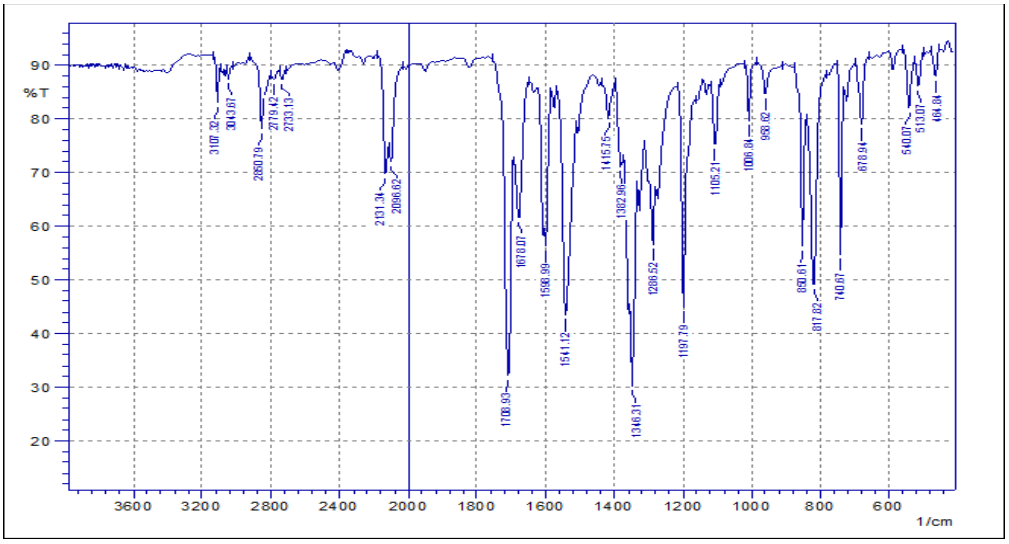


Figure3: IR Spectrum of compound T_1

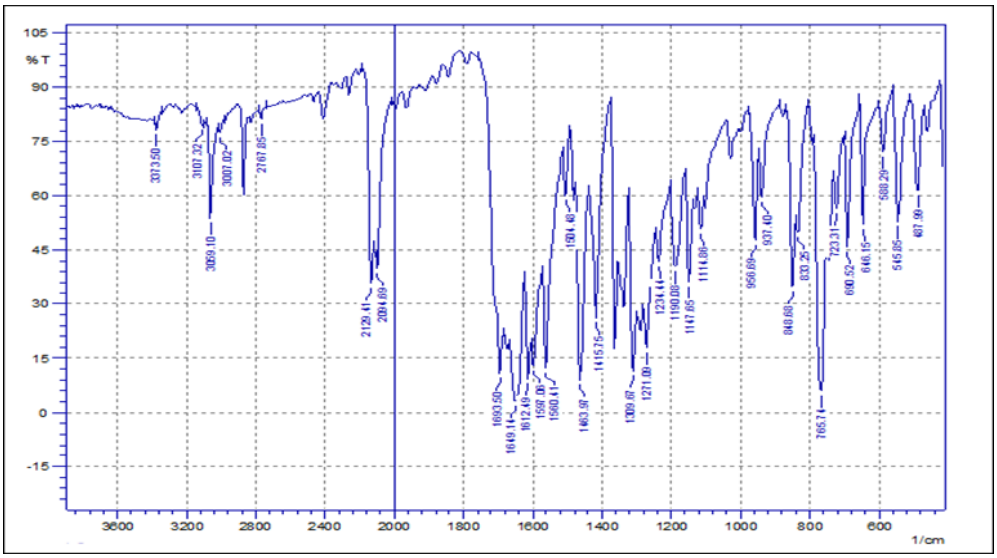


Figure 4: IR Spectrum of compound T_2

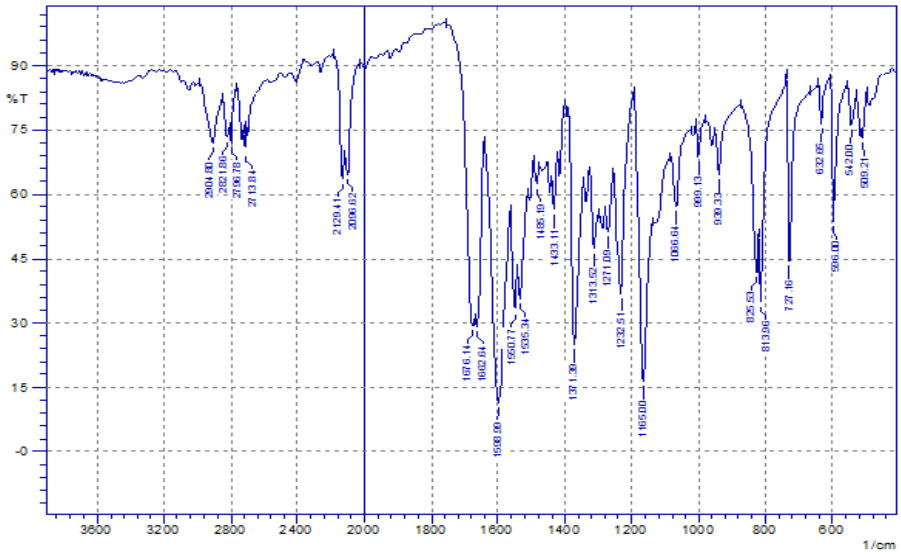


Figure5: IR Spectrum of compound T_3

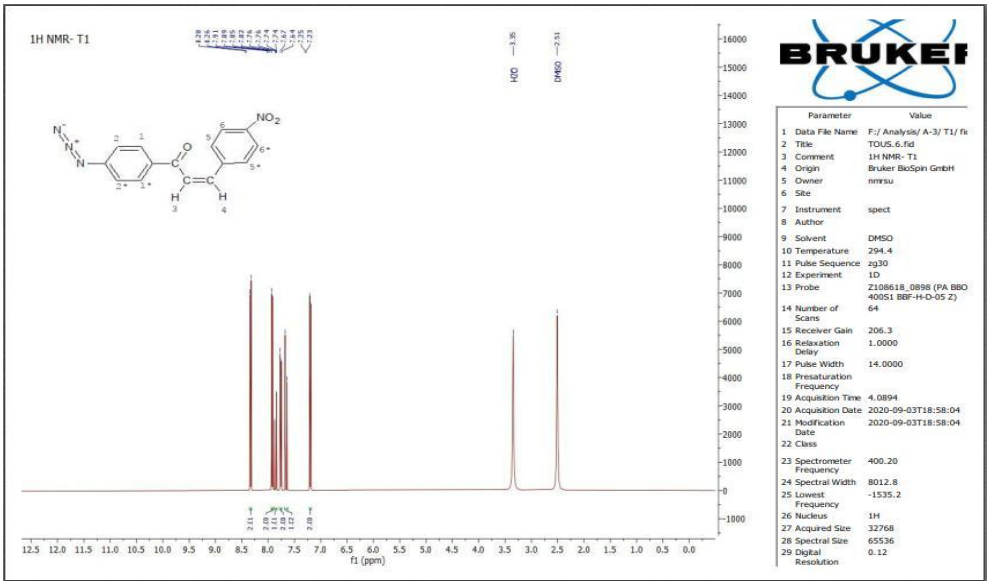


Figure 6 : Proton NMR Spectrum of compound T_1

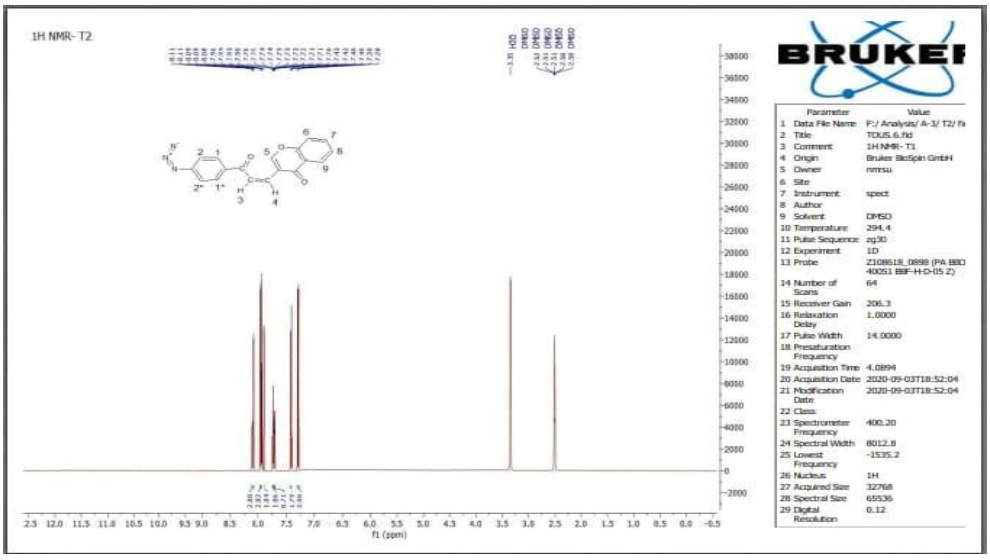


Figure7: Proton NMR Spectrum of compound T_2

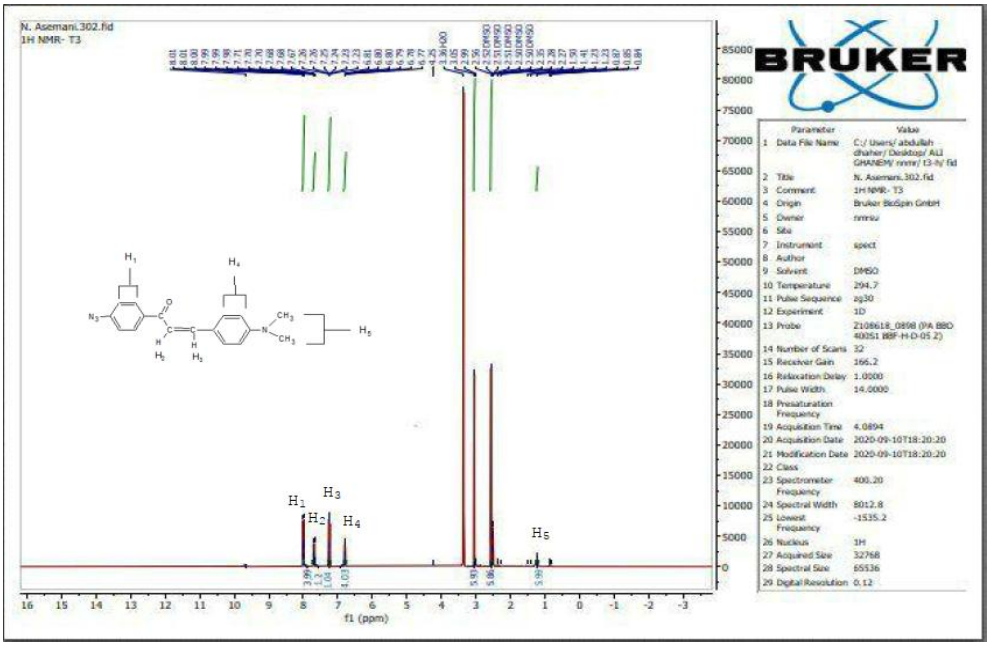


Figure 8: Proton NMR Spectrum of compound T_3

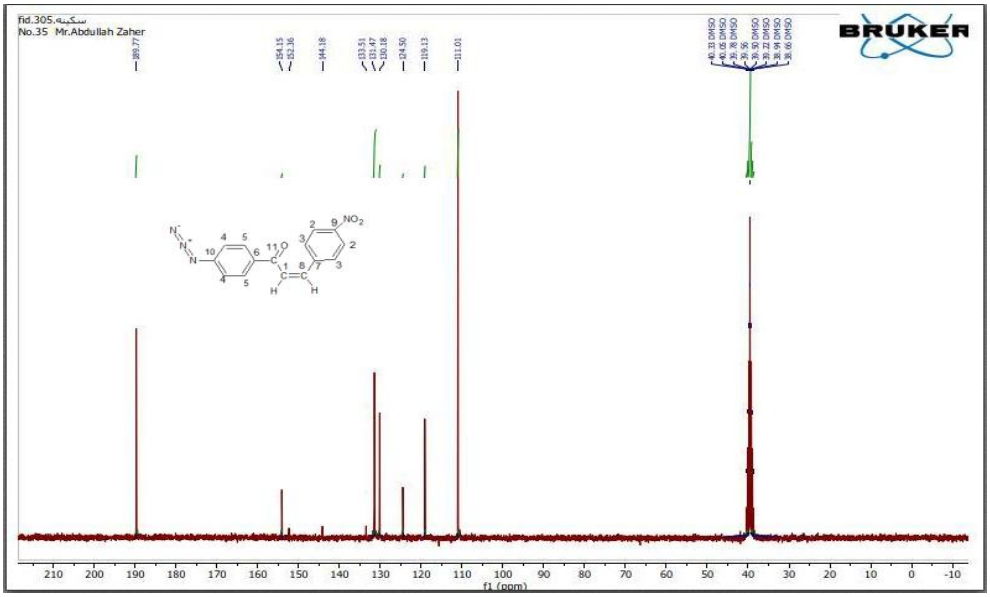


Figure 9: Proton ^{13}C-NMR Spectrum of compound T_1

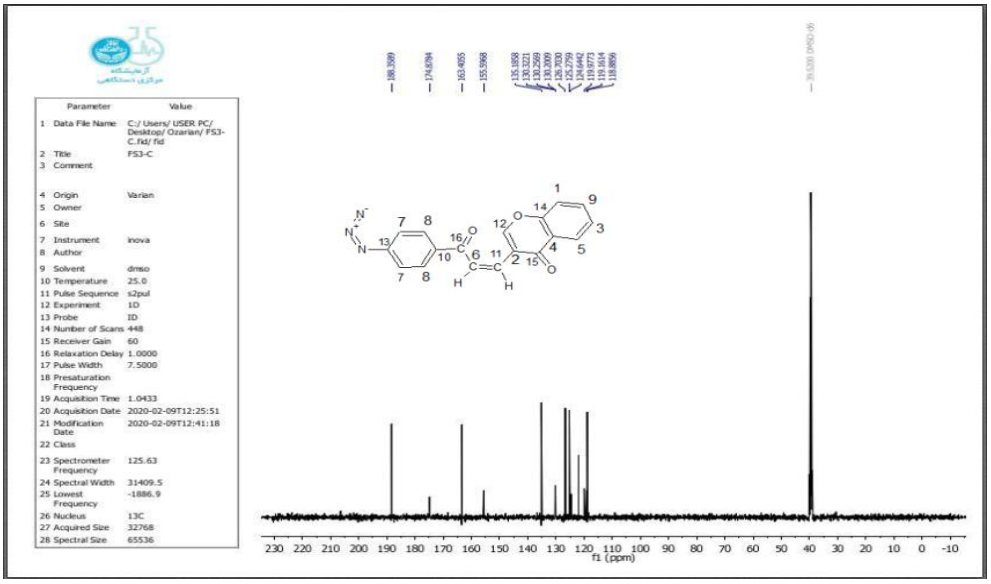


Figure10: Proton 13C-NMR Spectrum of compound T_2

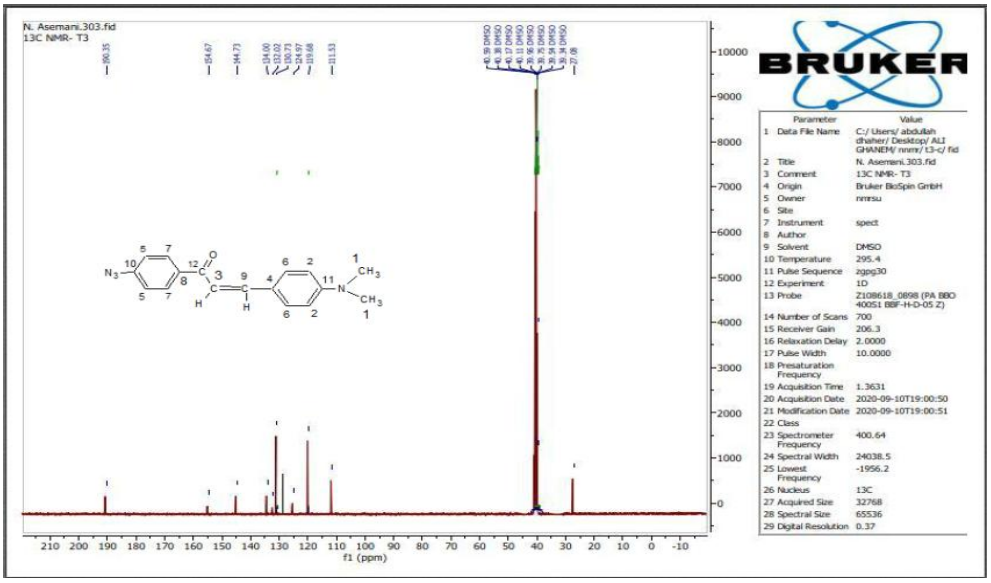


Figure11: Proton 13C-NMR Spectrum of compound T_3

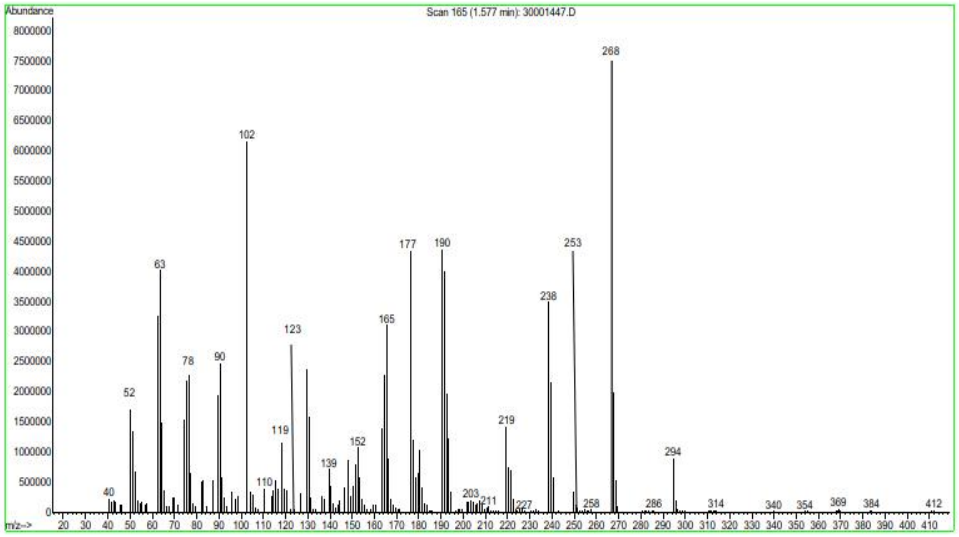


Figure12. : Mass spectra for T_1

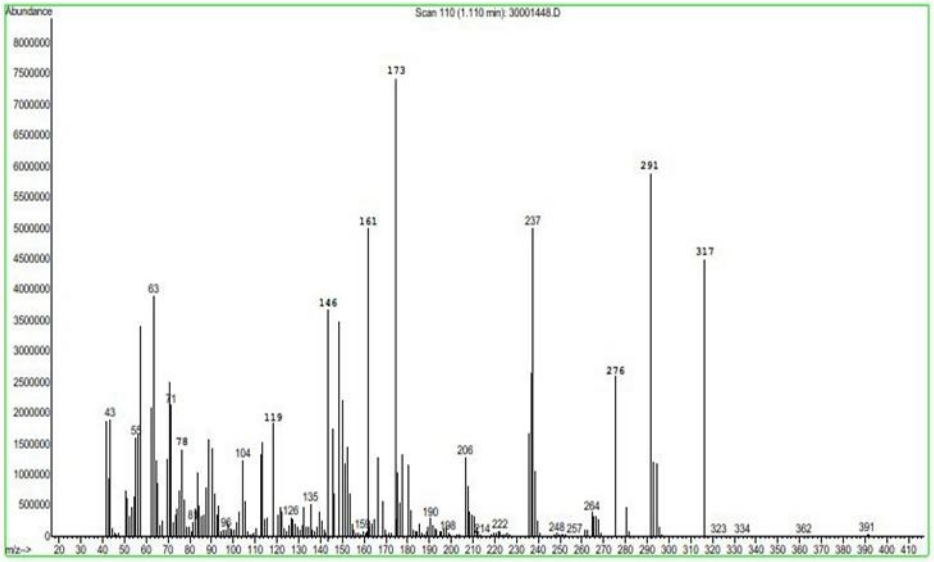


Figure13. : Mass spectra for T_2

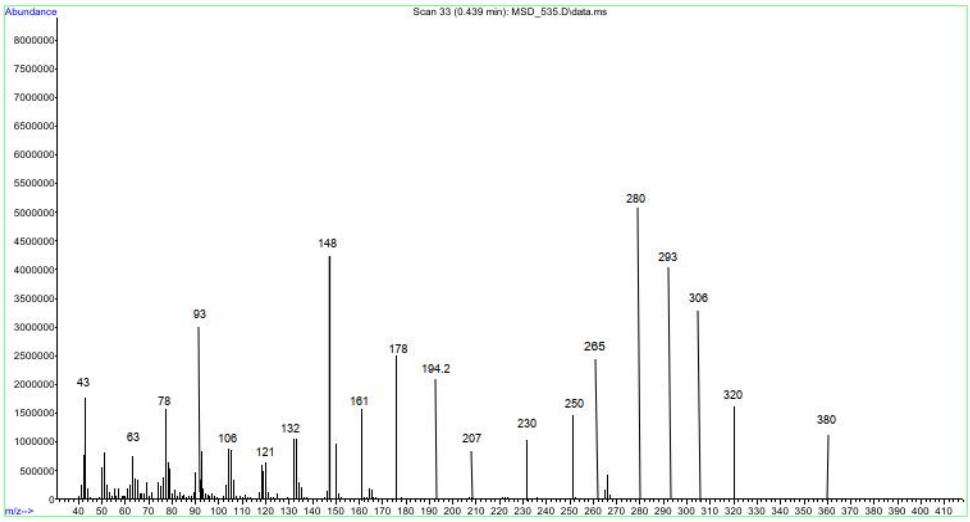
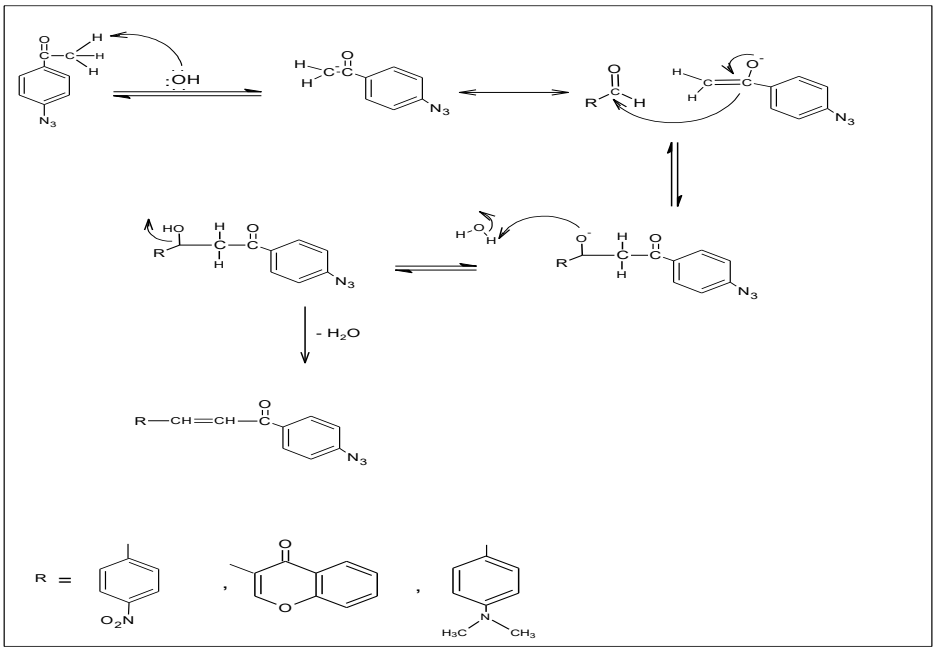


Figure14: Mass spectra for T_3

The compounds (T1, T2, and T3) were prepared through the reactions of the addition of 1-4-azidophenyl ethanone with some aldehydes using ethanol or, chloroform as a solvent in a basic medium in addition to the use of hydrochloric acid with a follow-up reaction and the figure (1) illustrates the preparation of Chalcones compounds fromazide. [16]



Scheme 1: The mechanism of preparing the Chalcones

Mechanism of linking the prepared azide compounds with the liposomes

The prepared compounds were coated with liposomes, based on a bonding method of triphenylphosphine with T1, T2, and T3, as the prepared organic compounds reacted. In the liposomes, it is selectively formed to form amide bonds, and binding occurs with high productivity and temperature Chamber in a suitable solvent, without any catalyst in chloroform solvent and without any catalyst;[17,18.19]Figure 15

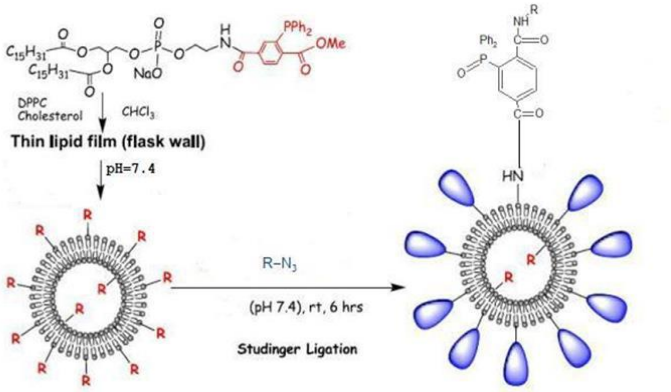


Figure 15 : Mechanism of binding organic compounds with liposomes

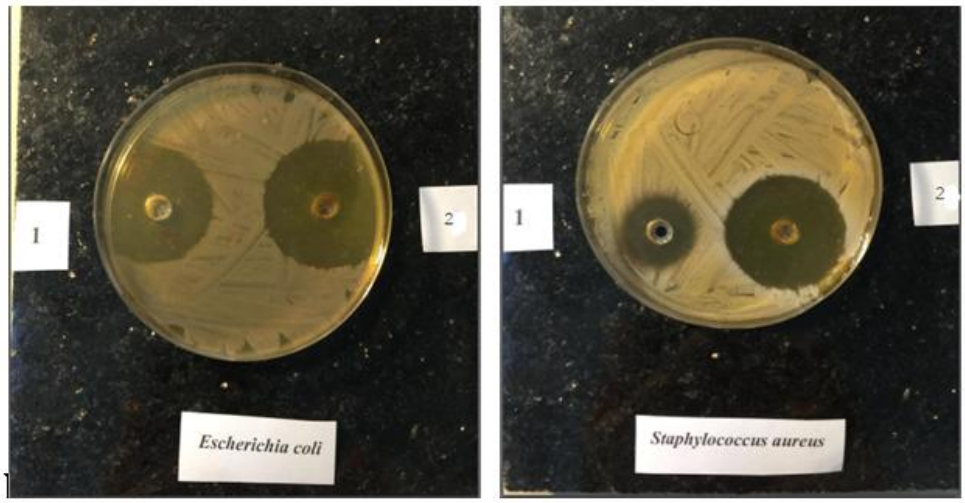
Applications

Antibacterial activity

The antibacterial [22, 23] activity of compounds was tested by agar – well diffusion method. Besides the antibacterial activity, Petri dishes with 20 ml of Mueller – Hinton agar were prepared, inoculated with 1x106 cell/ml (0.1 optical density on 540 nm wavelength), 100 µ of a 24 hours broth culture of test bacteria. Discs 6 mm in diameter each were made and filled with 100 µl of (150 mg/ml) extracts. The inoculated plates were incubated for 24 hours at 37°C. After incubation, the diameters of the inhibition zone diameter were measured in mm (Perez et al.,1990).The results of the examination against bacteria and fungi appeared were shown in Table (2) and the following figures [19.20,21,22]

Table(2): The diameter of the coil is indicated in mm for some of the organic compounds prepared

NO	Code	Conc. Mg/ml	Inhibition zone(mm) *S.aureus*	*E.coli*	*C.albicans*
1	T_1	100	24	35	50
2	T_2	100	35	40	50
3	T_3	100	15	20	20



Figure(16) : Demonstrates the biological activity of the compounds T1, and T2 against *E.coli*and *Staphylococcus aureus*

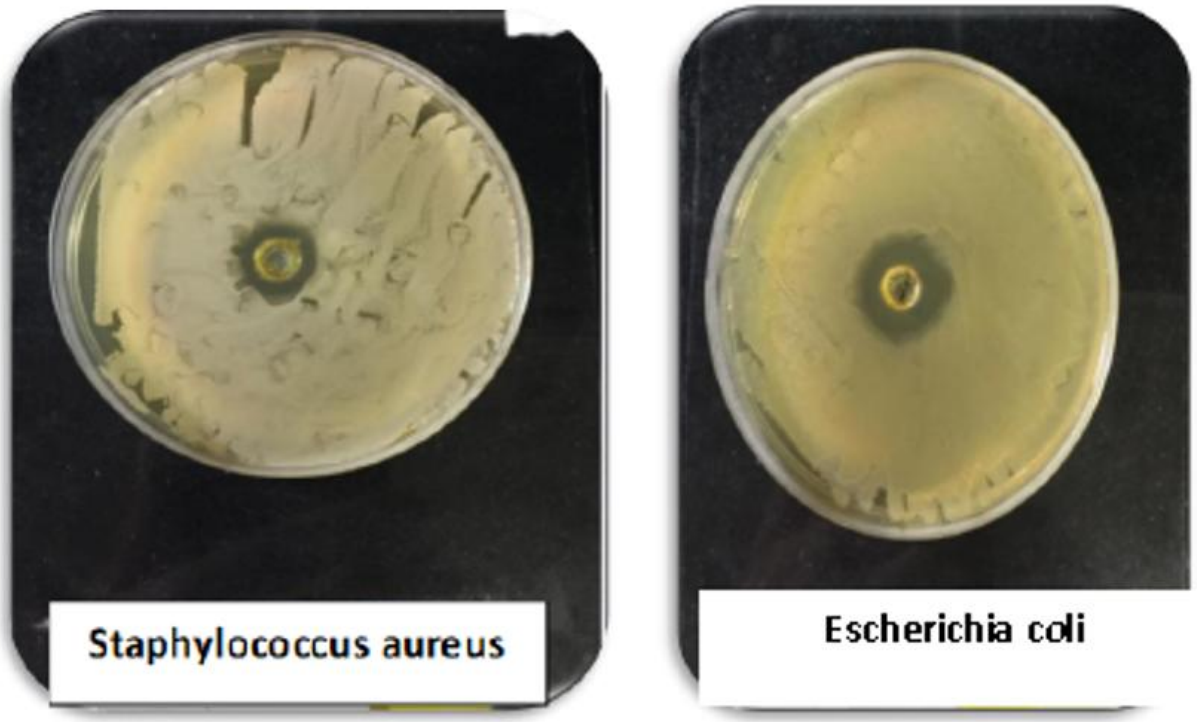


Figure 17:Demonstrates the biological activity of the compounds T3 against *E. coli and Staphylococcus aureus.*

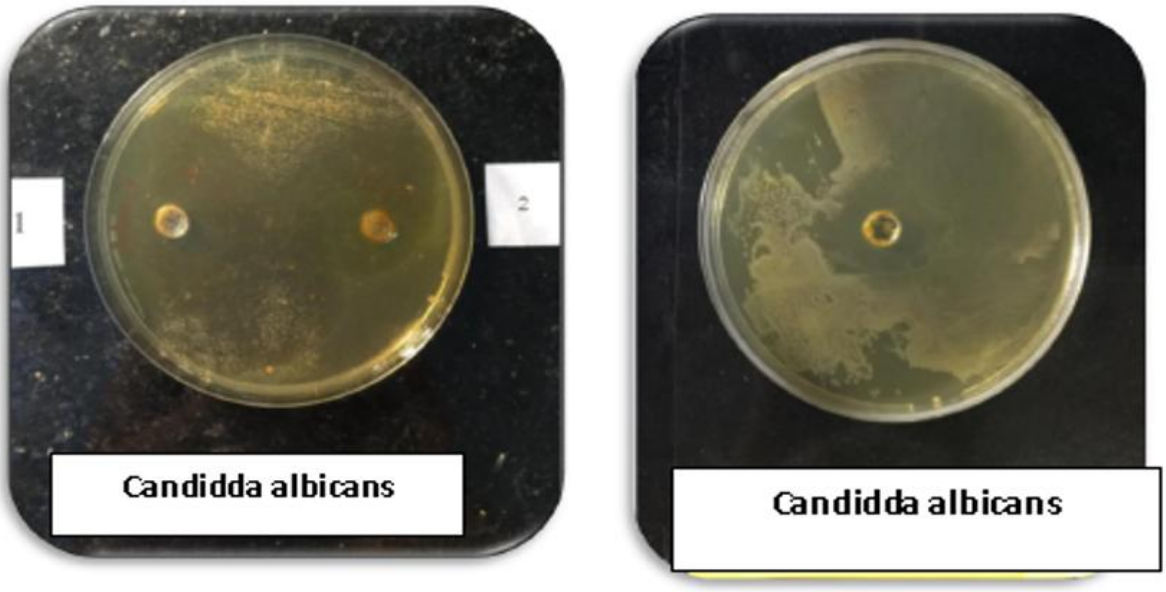


Figure18:Demonstrates the biological activity of the compounds T1,T2,T3 Against *fungicide Candida*

1. Evaluation of compounds prepared as antioxidants:

The results of free radicals present in the cell HCAM showed that the ROS level was 0.46 ng/ml in the untreated human cells, but it decreased in all the treated cells in the prepared compound T1 by 0.27ng / ml, while the second prepared compound did not show T2 activity (0.0ng / ml). The third compound showed T3 (0.23). Figure 19.

Table 3:Shows the effectiveness of the prepared compounds against oxidative stress on the cell line HCAM

Sample	**ROS concentration**	**Unit**
Control	0.46	ng/ml
T_1	0.27	ng/ml
T_2	0.0	ng/ml
T_3	0.23	ng/ml

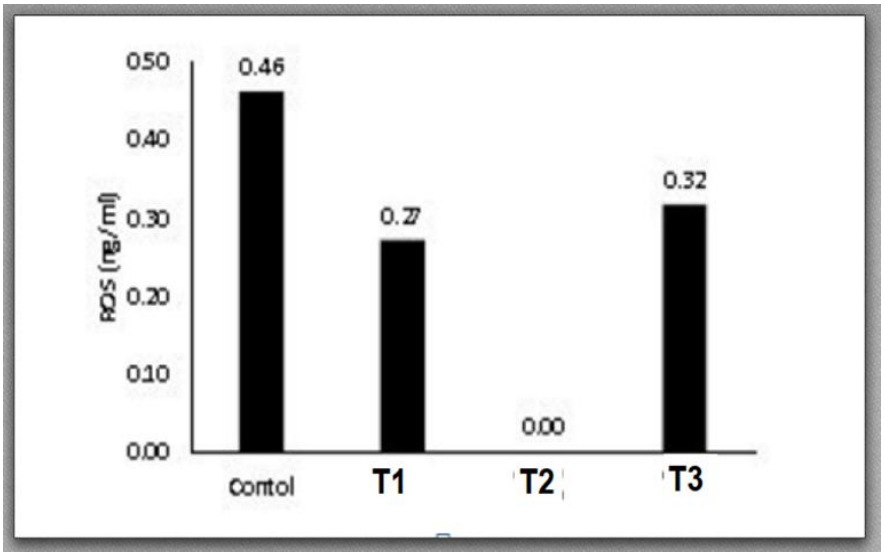


Figure 19: Shows the level of effectiveness of the prepared compounds against oxidation

5.3 Study of the anticancerocyte activity of compounds LT1, LT2, and LT3

The effectiveness of liposomal-bearing compounds against liver cancer (HCAM) has been studied. The results of the preliminary examination of the effectiveness of the compounds are shown in Figure 19. MTT was examined in the laboratory using a dye technique. It has a high inhibitory effect against cancer cells.

Cytotoxic Activity of T1, T2, and T3 Compounds Against Cancer Cells

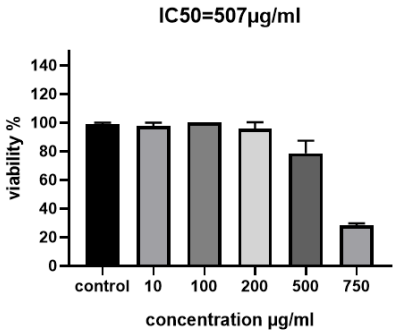
The cytotoxic activity of T1, T2, and T3 compounds was evaluated against cancer cells. T1 exhibited an inhibitory activity of 40.4% at a concentration of 1000 mg/mL after 72 hours of treatment. The IC50 value of T1 was determined to be 507 µg/mL, with a corresponding inhibition of 20%. Furthermore, the LC50 value of T1 was found to be 750 µg/mL, resulting in 40% inhibition. In comparison, T2 and T3 compounds showed inhibitory activities of 32.5% and 38.1%, respectively, at a concentration of 100 mg/mL. The IC50 value of T3 was calculated to be 381 µg/mL.

Table 4: Inhibition rate for liposome-loaded compoundsT1, T2 and T3 at aconcentration of 1000µg /ml

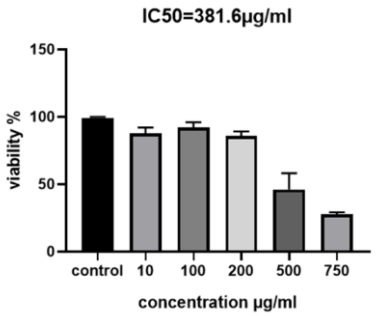
Concentrations 1000 µg/ml	T1	T2	T3
Mean %Inhibition	40.4	32.5	38.1

Table(5) of IC5 vitality rate for five vehicle-loaded liposomal concentrations T1, T3

Compound	Concentration µg/mL					IC50 µg/mL
	10	100	200	500	750	
	Viability %					
T_1	97.7	100	96.2	78.2	28.3	507
T_3	87.5	92	85.5	45.8	27.5	381



Figure(20) The half-lethal concentration of IC50



Figure(21) The half-lethal concentration of IC50

On the compound LT1 HCAM cell lineon the compound LT3 HCAM cell line

5.4 Transmission Electron Microscopy (TEM)

Electron microscopy imaging of the samples was used and liposomes were compared to none Compounds prepared with liposomes .In the presence of the compounds prepared, we observed the particles Liposomes are surrounded by prepared organic compounds. This indicates their presence inside the liposome(56.81- They in turn surround the drug, and the size of the nanoparticles ranges between(56.81,87.40,113.6)nm.

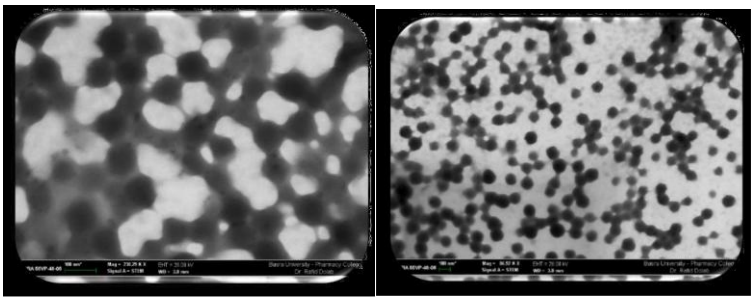


Figure 22: liposomes in the absence of prepared compounds

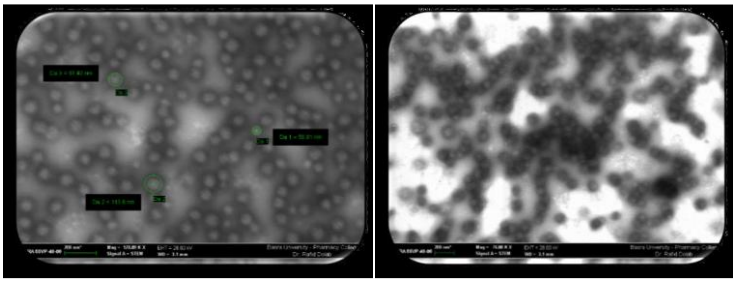


Figure23. liposomes with prepared compound

5.6 Study of the effect of the acidic function on the release of prepared compounds from the particle layers Lipid nanoparticles:

From T1 and T2 liposomes, the effect of change in acidic function on the release velocity of compounds was studied as the concentration of the material released was determined during different time periods. The concentration of the released substance was measured at different acidic functions, pH=7.2, ~~and~~ the default gastric solution SGF, and a hypothetical bowel solution SIF.

Time(hr)	pH=7.2	pH=1.2	pH=8.2
0	0	0	0
1	2.23	2	1.94
2	2.6	2.16	2
3	2.83	2.3	2.13
4	3.27	2.6	2.23
	3.57	2.97	2.38
7	4.3	3.34	2.6
9	5.57	4.53	3.34
11	7.05	5.9	4.46
13	8.9	7.64	5.94
15	10.75	9.27	7.47
24	14.46	11.49	10.1

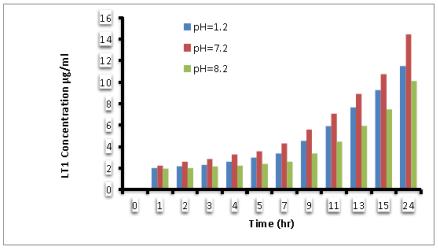


Figure24 T1concentrations released within 24 hours at pH=7.2 ,pH=1.2 .pH=8.2

Time(hr)	pH=7.2	pH=1.2	pH=8.2
0	0	0	0
1	7.75	4.24	6.43
2	9.5	8.62	8.62
3	12.57	13	12.57
4	15.2	17.3	16.9
5	20	26.17	21.7
7	27.2	34.94	28.8
9	35.8	43.7	35.8
11	45.9	52.48	43.7
13	56.87	63.4	54.67
15	70	74.4	67.89
24	86.69	86.7	83

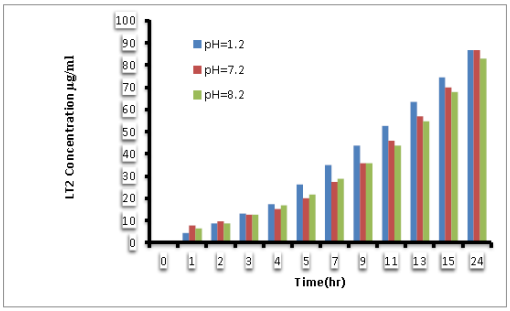


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Figure25 T2 concentrations released within 24 hours at pH=7.2 ,pH=1.2 .pH=8.2

References

1. Pauling L. The Nature of the Chemical Bond. 3rd ed. Ithaca, New York: Cornell University Press; 1969.

2. Jia-Wang Li, Jia-Cheng Li, Rui Zhang, Dewen Dong., Optimizing the Catalytic Performance of Amine-Functionalized 4CzIPN Derivatives by Investigation of the Twisted Intramolecular Charge Transfer Effect, The Journal of Organic Chemistry 2025, 90 (32) , 11636-11649.

3. Rucheng Zhao ,Yusheng , yuequn zhang , Jiabao Ling , Xinxin Liu, Jiaqi Xiang, Xiangchao Zeng & Tianfeng Chen . **Designing anticancer combretastatin A-4 analogues with aggregation-induced emission characteristics ,** pages 694-698(2022)

4. Massarotti A, Aprile S, Mercalli V, Del Grosso E, Grosa G, Sorba G, Tron GC. Are 1,4- and 1,5-disubstituted 1,2,3-triazoles good pharmacophoric groups? ChemMedChem. 2014 Nov;9(11):2497-508. doi: 10.1002/cmdc.201402233. Epub 2014 Jul 30. PMID: 25079879.

5. Naval Kapuriya , Rajesh Kakadiya , Mahesh M savant . Indian Journal of Chemistry -Section B Organic Chemistry including Medicinal Chemistry . vol.51B:1032–1038, July 2012

6. Anthony Tawiah , George Baffour pipim , Richard Tia, Evans Adei . Exploring the chemo-, regio-, and stereoselectivities of the (3 + 2) cycloaddition reaction of 5,5-dimethyl-3-methylene-2-pyrrolidinone with C,N-diarylnitrones and nitrile oxide derivatives: a DFT study . Journal of Molecular Modeling27(10), September 2021

7. Muhammad, B. B. M., & Al Badrani, K. A. A. A. (2022). Prepare several substituted pyridines derived from Chalcone and evaluate their bioactivity. International Journal of Health Sciences, 6(S5), 11556² 11564.

8. Singh P, Anand A, Kumar V. Recent developments in biological activities of chalcones: a mini review. Eur J Med Chem. 2014 Oct 6;85:758-77. doi: 10.1016/j.ejmech.2014.08.033. Epub 2014 Aug 12. PMID: 25137491.

9. Abdul-Reda N. A, Abdul-Ameer S. R. Synthesis, Identification and Biological Activity of Some New Chalcone derivatives from 8-Chlorotheophylline. Orient J Chem 2018;34(1).

10. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome: classification, preparation, and applications. Nanoscale Res

Lett. 2013 Feb 22;8(1):102. doi: 10.1186/1556-276X-8-102. PMID: 23432972; PMCID: PMC3599573..
11Bozzuto G, Molinari A. Liposomes as nanomedical devices. Int J Nanomedicine. 2015 Feb 2;10:975-99. doi: 10.2147/IJN.S68861. PMID: 25678787; PMCID: PMC4324542.
12. Maherani B, Arab-Tehrany E, Mozafari MR, Gaiani C,. Liposomes: A Review of Manufacturing Techniques and Targeting Strategies Linder M. Curr Nanosci. 2011;7(3):436-452.
13. Banerjee R, Tyagi P, Li S, Huang L. Anisamide-targeted stealth liposomes: a potent carrier for targeting doxorubicin to human prostate cancer cells. Int J Cancer. 2004 Nov 20;112(4):693-700. doi: 10.1002/ijc.20452. PMID: 15382053.
14. Kumar D, Singh S. Curr Org Chem. 2020;24(16):1853-1876.
15-Zhang H. Thin-Film Hydration Followed by Extrusion Method for Liposome Preparation. Methods Mol Biol. 2023;2622:57-63. doi: 10.1007/978-1-0716-2954-3_4. PMID: 36781749..
16. Tai Y, Pei S, Wan J, Cao X, Pan Y. Fragmentation study of protonated chalcones by atmospheric pressure chemical ionization and tandem mass spectrometry. Rapid Commun Mass Spectrom. 2006;20(6):994-1000. doi: 10.1002/rcm.2404. PMID: 16479556.
18. Breijyeh Z, Jubeh B, Karaman R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules. 2020 Mar 16;25(6):1340. doi: 10.3390/molecules25061340. PMID: 32187986; PMCID: PMC7144564.
19. Prescher, J., Dube, D. & Bertozzi, C. Chemical remodelling of cell surfaces in living animals. Nature 430, 873–877 (2004). https://doi.org/10.1038/nature02791
20. Machado AR, Assis LM, Costa JAV, Badiale-Furlong E, Motta AS, Micheletto YMS *et al*., Application of sonication and mixing for nanoencapsulation of the cyanobacterium *Spirulina platensis* in liposomes. *Int Food Res J* **21**: 2201–2206 (2014).
21. Wang Y, Xie Y, Li J, Peng ZH, Sheinin Y, Zhou J, Oupický D. Tumor-Penetrating Nanoparticles for Enhanced Anticancer Activity of Combined Photodynamic and Hypoxia-Activated Therapy. ACS Nano. 2017 Feb 28;11(2):2227-2238. doi: 10.1021/acsnano.6b08731. Epub 2017 Feb 6. Erratum in: ACS Nano. 2019 Apr 23;13(4):4855. doi: 10.1021/acsnano.9b01888. PMID: 28165223; PMCID: PMC5332348.
22. Prescher, J., Dube, D. & Bertozzi, C. Chemical remodelling of cell surfaces in living animals. *Nature* **430**, 873–877 (2004). https://doi.org/10.1038/nature02791

تخليق وتوصيف بعض مركبات الأزيد المحملة على الليبوسومات ودراسة فعاليتها كمضادات للأكسدة ومضادات للسرطان

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الملخص: شملت الدراسة تحضير ثلاثة أنواع من مركبات الكالكون (T3، وT2، T1) من خلال تفاعل إضافة (4-أزيدوفينيل إيثانون) مع بعض أنواع الألدهيدات (أوكسوكرومان-3-كربوليدهيد، 4-ثنائي ميثيل أمينو بنزالدهيد، 3-نيترو بنزالدهيد). في تقنية (13C-NMR). ، وتقنية الرنين النووي المغناطيسي للكربون-13(1H-NMR) الرنين النووي المغناطيسي للبروتون (FTIR) وجود وسط قاعدي، تم تشخيص المركبات المحضرة باستخدام تقنية الأشعة تحت الحمراء بتحويل فورييه ، وأظهرت نتائج جيدة. حُمّلت HCAM مطياف الكتلة. دُرست فعالية هذه المركبات ضد الإجهاد التأكسدي على خط خلايا مركبات الأزيد المُحضّرة على جسيمات دهنية مُحضّرة من الليسيثين. كما دُرست تأثيرات الحموضة على تحرير المركبات المُحضّرة من طبقات الجسيمات النانوية الدهنية في بيئات مختلفة: بيئة ذات درجة حموضة 7.2، وبيئة معدية (درجة حموضة 1.2)، وبيئة معوية (درجة حموضة 8.2)، وذلك باستخدام مطيافية الأشعة فوق البنفسجية عند درجة حرارة 37 درجة مئوية. ، ومعدل التثبيط عند تركيز HCAM 1000 قُيست فعالية الجسيمات الدهنية المُحمّلة ضد خلايا سرطان الكبد البشري باستخدام تراكيز مختلفة (10، 100، 200، 500، 750) (IC50) ميكروغرام/مول، وحُسب تركيز نصف التثبيط المميت كمذيب. أظهرت النتائج فعالية عالية. كما تم إجراء المقارنة (DMSO) ميكروغرام/مول، باستخدام ثنائي ميثيل سلفوكسيد للجسيمات الشحمية في وجود المركبات المحضرة وغيابها (TEM) باستخدام مجهر إلكتروني نافذ.