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New Indole compounds with acidic terminal

Israa N. Hamdi[1]*, Salih M. Salman[2], luma S. Abd[3]

[1,3]University of Diyala, College of Sciences, Department of Chemistry, Diyala, Iraq

[2]University of Diyala, College of Medicine, Department of Chemistry, Diyala, Iraq

scichems2103@uodiyala.edu.iq

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Abstract

Indoles are important heterocyclic molecules modified by many researchers to a lot of derivatives used in all the fields of the life including medical, therapeutic and industrial application. This work is continuous effort to what was began before aiming to the synthesis of benzo indole derivatives by coupling with some amino acids core including alanin, valine and tryptophanvia Schiff reaction in order to find new potential anticancer agents. Schiff reaction happen between aldehyde and amine groups so that benzo indole (1, 1, 2-trimethyl-1H-benzo[e]indole) (1) need a instead of to functionalization to create two aldehyde groups by treatment with phosphoryl chloride (POCl3), this treatment yield the precursors 2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) malonaldehyde (2), which is capable of applying Schiff reaction. The 3D investigation of the precursors shows the aldehyde group close to the methyl group is under steric hindrance, so that it needs longer time for coupling with amino acids from the other aldehyde group. This property allow the control of the coupling,there for it is possible to synthesize both the coupling so that we could synthesis both mono and di-substituted through the reaction of the precursors 2-(1, 1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) malonaldehyde (2) with each mentioned aromatic amines. As a results, four pure compounds have been synthesized and characterized using both 1H and 13C NMR spectroscopy. Two of the products are proven to have a good ability in a human liver cancer

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cell line HepG2 inhibition at the optimize temperature degree 37Cº within the 24 hours period which makes them a potential anticancer agents.

Keywords: malonaldehyde, Schiff bases, Anticancer; HepG2; steric hindrance

مركبات الإندول الجديدة ذات الطرف الحمضي

إسراء ناظم حمدي[1] ، صالح مهدي سلمان[2] ، لمى سلمان عبد [3]

[1,3] جامعة ديالى - كلية العلوم - قسم الكيمياء

جامعة ديالى - كلية الطب - قسم الكيمياء

الخلاصة

الإندولات هي جزيئات حلقية غير متجانسة مهمة تم تعديلها من قبل العديد من الكيميائيين إلى الكثير من المشتقات المستخدمة في جميع مجالات الحياة بما في ذلك التطبيقات الطبية والعلاجية والصناعية. هذا العمل هو جهد مستمر لما بدأ قبل أن يهدف إلى تخليق مشتقات benzo indole عن طريق الاقتران ببعض الأحماض الأمينية الأساسية بما في ذلك alanin و valine و tryptophan عبر تفاعل Schiff من أجل إيجاد عوامل محتملة جديدة مضادة للسرطان. يحدث تفاعل شيف بين مجموعتي الألدهيد والأمين بحيث يحتاج البنزو إندول (1 ، 1 ، 2-ثلاثي ميثيل H-benzo [e] indole1) [1] إلى التفعيل لإنشاء مجموعتين من الألدهيد عن طريق المعالجة باستخدام كلوريد الفوسفوريل (3POCl) ، وهذا ينتج عن العلاج السلائف 2-(1،1-malonaldehyde-1H-benzo [e] indol-2 (3H) -ylidene) dimethyl [2] ، وهو قادر على تطبيق تفاعل شيف. يُظهر التحقيق ثلاثي الأبعاد للسلائف أن مجموعة الألدهيد القريبة من مجموعة الميثيل تخضع لعائق فاصل ، بحيث تحتاج إلى وقت أطول للاقتران مع الأحماض الأمينية من مجموعة الألدهيد الأخرى. تسمح هذه الخاصية بالتحكم في الاقتران حتى نتمكن من تصنيع كل من الأحادي والثنائي المستبدل من خلال تفاعل السلائف 2- (1 ، 1-ثنائي ميثيل -1 H-benzo [e] indol-2 (3H) -ylidene) malonaldehyde [2] مع كل الأمينات العطرية المذكورة. نتيجة لذلك ، تم تصنيع أربعة مركبات نقية وتميزت بالطيف الطيفي H1 و C NMR13. تم إثبات أن اثنين من المنتجات يتمتعان بقدرة جيدة في تثبيط خط خلايا سرطان الكبد البشري 2HepG عند درجة الحرارة المثلى 37 درجة مئوية خلال فترة 24 ساعة مما يجعلها عوامل محتملة مضادة للسرطان.

الكلمات المفتاحية: malonaldehyde,قواعد شيف, مضاد للسرطان,HepG2, عوائق فراغية.

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Introduction

In general heterocyclic compounds are an important class of organic chemistry, which can be defined as compounds that have other atoms beside carbon and hydrogen such as oxygen, nitrogen and silver. They are too many compounds including indoles. Benzo indole (1, 1, 2-trimethyl-1H-benzo[e]indole) is an important derivative of indoles. There are a lot of modifications applied to for this compound in order to find useful application in all fields of the life. (1, 1, 2-trimethyl-1H-benzo[e]indole) was modified and used as fluorescent probes for the imaging of tumors in vivo [1-5], In our daily lives, heterocyclic compounds has great interest. Heterocyclic compounds can be used in a variety of ways. Pharmaceuticals, agrochemicals, and veterinary items are the most common applications. They are also used as sanitizers, developers, antioxidants, corrosion inhibitors, copolymers, and dye materials. They are used in the synthesis of other organic compounds as vehicles[6].The indole Schiff bases were known as a significant class of heterocyclic organic compounds which have wide applications in many fields for examples anti-inflammatory activity [7], antimicrobial activity, [8] antibacterial, antifungal, antitumor activity [9] and antioxidant [10]Schiff bases also called imines are characterized by the azomethine (-C=N-) group and are It usually formed by the condensation of an aldehyde or ketone with a primary amine. This condensation reaction between a carbonyl compound and primary amine leading to the configure of schiff base. Schiff's bases are generally excellent as chelating agents especially when they are a functional groups such as –OH or –SH . It is located near the isomethene groups to form a five or six-membered rings with the metal ion [11]. The use of Vilsmeier-Haack reagent (PoCl3 / DMF) has been documented to form a variety of aromatic and heterocyclic substrates well [12]. It can be applied to enter an aldehyde group to Aromatic compounds, but many other transformations can be achieved using this technique [13]. The reagent has also been widely used to induce various chemical conversions of another classes of compounds. Many of these reactions lead to new and convenient methods of synthesizing various heterocyclic compounds [14].

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Materials and Methods

Chemical and Solvents

Chemical, solvent and reagents used in experiments curried out in this study were from different sources and used without any further purification.

Spectrophotometry

Purification and verification of the final derivatives were performed using spectroscopic ways such as 1H and 13C NMR (Avance Neo Neo 400, Iran), FT-IR spectrophotometer Perkin-Elmer Spectrum version 10.02 at the Department of Chemistry, Faculty of Science, Diyala University.

Programs and Software

The chemical structure, schemes, 3D structure study was performed using ChemOffice Ultra 2006 (CambridgeSoft) and ChemSketch 2010 (Advanced Chemistry Development, lnc.).

Tools and Instruments

The reactions were followed throug was achieved through thin layer chromatography (TLC) using alumina plates (size 20×20 cm) percolated with silica gel and Fluorescence Analysis Cabinet Model CM-10 as a detector. The Stuart SMP10 electronic apparatus used to measure the melting point of the final products ((Department of Chemistry, Faculty of Sciences, and University of Diyala).

General Methods

Functionalization Reaction: 1,1,2-trimethyl-1H-benzo[e]indole (1 eq.) (1) dissolved in anhydrous dimethyl form amide (DMF) 15 mL. Then 15 mL phosphoryl chloride solution (POCl3) in DMF gradually added to the first solution during half hour at 4 °C. The later was refluxed at about 90 °C for at least or about than 4 h, TCL (3:1) hexane: ethyl acetate showed the consumption of 1,1,2-trimethyl-1H-benzo[e]indole (1 eq.) (1), the final mixture poured into ice water, neutralized by sodium hydroxide (35% NaOH). Products precipitated, filtered off,

washed with water, and dried in a 60 °C oven to produce yellow crystals of 2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) malonaldehyde(2) .[15-17]. First Coupling Reaction: One equivalent of 2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) malonaldehyde 2 dissolved in EtOH (15 mL) were mixed with one equivalent of amino acids solution in 15 mL EtOH. 1 mL of glacial acetic acid then gradually added and refluxed. TLC 3:1 hexane: ethyl acetate showed the dissapearance of the starting material after 15 hours. The mono-substituted indole derivative precipitates after solvent removal, are filtered, washed, and dried. Two new compounds were synthesized using the procedures of this step: -

2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)propanoic acid (3)

2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) malonaldehyde (2) (0.20 g, 0.000754 mmole) was reacted with alanine according to first coupling procedure to dark yellow crystals of 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)propanoic acid (3) (For more details see figures 1 and 2).

2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)-3-methylbutanoic acid (4)

2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) malonaldehyde (2) (0.20 g, 0.000754 mmole) was reacted with Valine according to first coupling procedure yield brouwn crystals of 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)-3-methylbutanoic acid (4)(For more details see figures 3 and 4).

ؽ Second Coupling of Compound 4 with amino acids: One equivalent of the mono-substituted indole derivatives 4 or 5 in 15 mL CH3CH2OH and one equivalent of aliphatic amine solution in 15 mL CH3CH2OH were mixed. One mL of glacial acetic acid was added gradually to the first mixture and refluxed. TLC 3:1 hexane: ethyl acetate showed the consumption of the starting material after 24 hours. The solvent was removed then the di-substituted derivative of indole was precipitated, filtered, washed, and dried [18]. Two syntheses were applied on applying this step: -

2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)propane-1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-methylbutanoic acid) (5)

2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)-3-methylbutanoic acid (4) (0.20 g, 0.000754 mmole) was reacted with Valine as according to in the first coupling procedure to yield yellow crystals of 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)-3-methylbutanoic acid (5) (For more details see figures 5 and 6).

2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) propane-1,3-diylidene) bis (azan-1-yl-1-ylidene) bis (3-(1H-indol-3-yl) propanoic acid) (6)

2-(((1E,2E)-2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-oxopropylidene)amino)-3-(1H-indol-3-yl)propanoic acid (0.20 g, 0.000754 mmole) was reacted with Tryptophan as according to in the first coupling procedure to yield greenish brown crystals of 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene) amino)-3-methylbutanoic acid (6) (For more details see figures 7 and 8).

HepG2 Cell Line Investigation

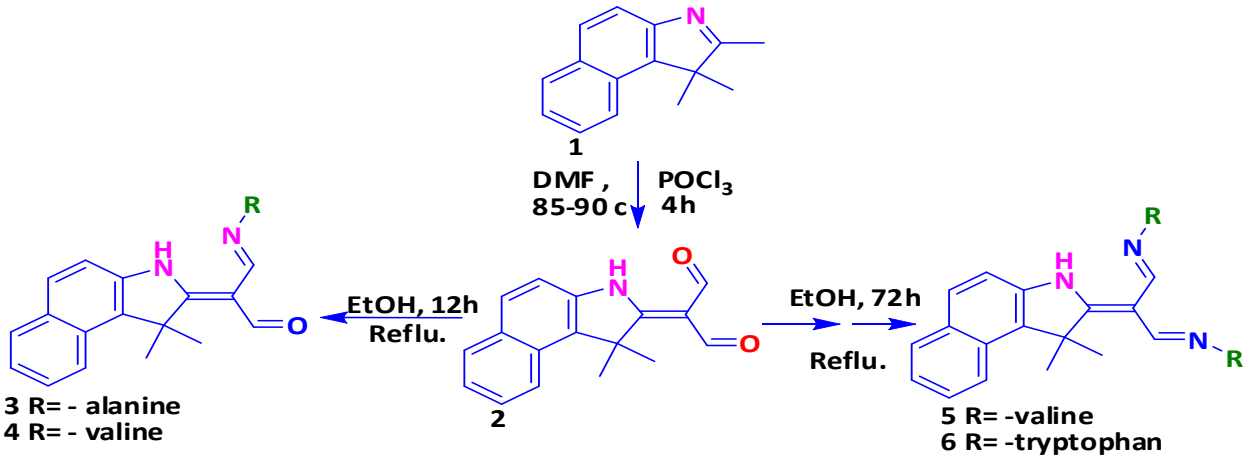
Solutions used in this experiment (Culture Media RPMI-1640, Trypsin–Versin solution, Crystal Violet Stain and Trypan blue stain) were prepared according to Giuliano method [19]. The HepG2 Cell Line and normal cell Line (Rhabdomyosarcoma) (RD) was grown in RPMI-1640 medium equipped with 10% calf fetal serum according to the Freshney method [20-21]. The cytotoxicity assay was carried out using the crystal violate stain according to the method of Freshney [22]. The target compounds were dissolved in DMSO and diluted by serum free media (SFM) to prepare different concentrations range of (50,100) µg/mL. Two types of cell lines were used human liver cancer (HepG2), and normal human (RD)(RPMI-1640) cell lines. The tumor cells (1 x105 cell / mL) were seeded in 96-well microplate and incubated for 24 hours at 37Cº, and then old media was changed with new serum-free medium (SFM) containing concentrations of each compound. Plate was incubated for 24 hours in humidified incubator at 37 Cº containing 5% CO2. After incubation, the culture medium was discarded and 100 µl of

crystal violate was added to each, and re-incubated 20 min at 37 Cº. The results were read by using the ELISA with a wavelength of 492 nm. The inhibiting ratio was calculated according to the equation. Percentage of cell inhibition = (absorbance reading of control cells - absorbance reading of treated cells for each concentration/absorbance reading of control cells) x 100

Results and Discussion

Chemistry

The aim of this research is the synthesis of new benzo indole derivative and investigates their tautomer. The target synthesis is achieved by the Schiff's base reaction starting with 1,1,2-trimethyl-1H-benzo[e]indole (1) and some amino acids including Alanin, Valine and Tryptophan as shown in scheme-1.



In order to apply the Schiff's base reaction between 1,1,2-trimethyl-1H-benzo[e]indole (1) and aromatic amine, the starting material need modification to create and aldehyde group which can achieved easily by treatment of 1,1,2- trimethy l-1H-benzo [e] indole (1) with Phosphoryl chloride (POCl3) to produce the precursor 2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) malonaldehyde (2), which contain two aldehyde groups with can easily couple with the chosen amino acids compounds. The Schiff's base reactions under moderate conditions were applied on the precursor 2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene) malonaldehyde (2) aiming to synthesize the di-substituted benzo indole derivatives with mentioned amino acids, but instead of di-substituted benzo indole this reaction produced the unexpected mono-substituted derivatives even when applying one equivalent of precursor with two equivalent of aromatic

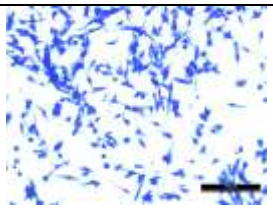
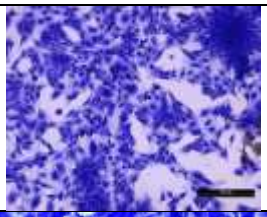
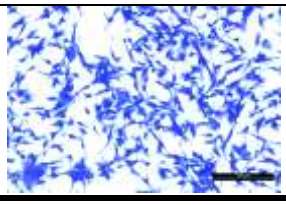
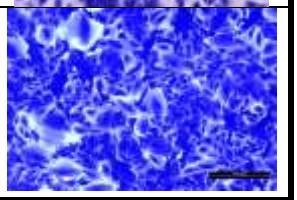
amine. As a results the reactions of 2-(1,1-dimethyl-1H-benzo[e] indol-2(3H)-ylidene) malonaldehyde (2) with Alanin and Valine produced the mono-substituted benzo 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e] indol-2(3H)-ylidene)-3-oxopropylidene) amino) propanoic acid(3) and 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene) amino)-3-methylbutanoic acid (4) respectively instead of the di-substituted derivatives 2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e] indol-2(3H)-ylidene) propane-1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-methylbutanoic acid) (5) and 2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)propane-1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-(1H-indol-3-yl)propanoic acid) (6) respectively. The 3D investigations of the intermediate show that the reasons for production of the mono derivatives instead of di-derivatives under short time reaction is the steric hindrance at the reaction center close to the di methyl of the indole moiety, which needs triple time required or the mono substituted of the first substitution. Therefore for the first 12h reaction the mono-substituted derivatives 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene) amino) propanoic acid (3) and 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene) amino)-3-methylbutanoic acid (4) can be easily separate as pure crystals using NMR. While the di-substitution derivatives 2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e] indol-2(3H)-ylidene) propane-1,3-diylidene) bis (azan-1-yl-1-ylidene) bis(3-methylbutanoic acid) (5) and 2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)propane-1,3-diylidene) bis(azan-1-yl-1-ylidene) bis(3-(1H-indol-3-yl) propanoic acid) (6) achieved and separated in NMR pure crystals after applying the same reaction for about 72h using the mono-derivatives with the same amine.

Anticancer Activity

Two synthesized compounds were investigated against cancer cell line as shown in table1. The investigated compounds show a good activity in a human liver cancer cell line HepG2 inhibition at the optimize temperature degree 37C^{o} within the period of 24 hours using two concentrations (50 µg/m and 100 µg/m) in comparison with the effects of the same compounds on normal cell line. The mono-substituted derivative of benzo indole- amino acids 2,2'-(1E,1'E)-(2-(1,1-dimethyl -1H-benzo [e]indol -2(3H)-ylidene) propane- 1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-methylbutanoic acid) (4) increase the power of inhibition of HepG2 cell line about 3.27 times in high concentration solution (50 µg/m, while in the low concentration solution they increase the rate of its activity in from about 1.48 time. The di-substituted analog of 2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e] indol-2(3H)-ylidene)propane-1,3-diylidene) bis(azan-1-yl-1-ylidene) bis (3-(1H-indol-3-yl)propanoic acid) (6) exhibit increasing in cell

cancer activity around 1.20 times in in the low concentration solution, but this inhibition rate reach to about 1.3 time times in high concentration solution.

Tabel 1: The Effect Synthesized Derivatives on Cancer and Normal Cell Line for 24h Exposures at 37 ºC

No	Derivatives	Inhibition ratiol100% Normal Cell Line Rd		Inhibition ratiol 100% Normal Cell Line Cancer HepG2		Cell line HepG2 in 100 µg/m Images	Normal Cell Line in 100 µg/m Images
		50 µg/m	100 µg/m	50 µg/m	100 µg/m		
1	4	10.11	12.76	32.56	76.36		
2	6	9.19	23.54	24.23	68.18		

Conclusion

Four new derivative of benzo indole were synthesized from 1,1,2-trimethyl-1H-benzo[e]indole after functionalization with POCl3 and coupling with three aromatic molecules including alanin, valine and tryptophan via Schiff reaction. This reaction subjected stearic hindrance and property already overcame by controlling the time of reactions. This property is directed and used to synthesis both mono and di-substituted derivatives. The synthesized compounds show a good ability for inhibition of HepG2 cancer cell line, which make them useful as potential anticancer agents.

Acknowledgments

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Supplementary Material

Spectroscopic data andy of synthesized compounds 3–6

2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)propanoic acid (3)

(0.12g, mmole, 48%) m.p. (180 C°).1H NMR chemical shifts at (400 MHz, DMSO-d6, δ in ppm): δ = 9.79 (s, 1H, -CH=O) ,7.93 (s, 1H, -CH=N-),7.49-8.21 (m, 6H, Ar-H), 3.80 (m, 0.70 , C=CH-OH) , 2.50 (d, 1H, =N-C(CH3)(COOH) , 1,78 ,1.94 (s, 6H, 2x CH3) and 1.38,1.1s (m,3H,CH3) . The 13C-NMR spectra for compound (3), shows the signals (400 MHz, DMSO-d6, δ in ppm): δ =179.67, for CH=O,172.04 (=CH-OH) for 137.87 for N-C, 133.01 for NH-CH=C,109.13 for (-C=C-), 122.88- 130.25 for Ar-C, 114.68 for O=C-C=C, 52.93 for CH3-C-CH3 ,48.71 for =N-C-(CH3)(COOH) 22.27 for 2× CH3) and 16.53, for (CH3).

2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)-3-methylbutanoic acid (4)

(0.1g, mmole, 37%) m.p. (160 C°).1H NMR chemical shifts at (400 MHz, DMOS-d6, δ in ppm): δ = 9.79 (s, 1H, -CH=O) ,7.93 (s, 1H, -CH=N-),7.51-8.20 (m, 6H, Ar-H), 3.51 (m, 1 , C=CH-OH) , 2.33 (d, 1H, =N-C(CH3)(COOH) ,2.13 –C(CH3)2 ,1,80 ,1.94 (s, 6H, 2x CH3) and 0.93 ,0.88 (m,6H,CH3) . The 13C-NMR spectra for compound (4), shows the signals (400 MHz, DMSO-d6, δ in ppm): δ =179.67, for CH=O,169.42 (COOH) for 137.89 for N-C, 133.01 for NH-CH=C,109.13 for (-C=C-), 122.88- 132.21 for Ar-C, 114.68 for O=C-C=C, 68.18 , -N-C , 52.93 for CH3-C-CH3, 29.82 for -C (CH3)2 , 22.27 for (2× CH3) and 19.62 for (2xCH3).

2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)propane-1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-methylbutanoic acid) (5)

(0.12g, mmole, 35%) m.p. (168C°). 1H NMR chemical shifts at (400 MHz, DMSO-d6, δ in ppm): δ =12.25 (m, 2H, COOH), 7.68 (s, 2H, -CH=N-), 7.32-8.02 (m, 6H, Ar-H, 3.088 (d, 2H, =N-CH, 3.04 (s-2H,CH2),and 1.91, 1.79 (s, 6H, 2xCH3 for indole), 1.51, 1.47 (s, 12H , 4xCH3 for acid part). The 13C-NMR spectra for compound (5), shows the signals (400 MHz, DMSO-d6, δ in ppm): δ =172.53 (COOH) for 135.97 for N-C , 129.85 for NH-CH=C, 85.95 for (-C=C-), 122.31- 129.85 for Ar-C, 118.24 for O=C-C=C, 52.93 or CH3-C-CH3, 24.63 for 4x -N- CH ,24.07 for –C(CH3), 22.59 for –C(CH3)2 , 21.60 for (2xCH3).

2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)propane-1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-(1H-indol-3-yl)propanoic acid) (6)

(0.12g, mmole, 39%) m.p. (197C°).1H NMR chemical shifts at (400 MHz, DMSO-d6, δ in ppm): δ = 12.17 (m, 2H, COOH), 11.07, 10.97 (s, 2H, NH of acid part) ,7.36 (s, 2H, -CH=N-), 6.68-8.19 (m, 16H, Ar-H, 3.00 (d, 2H, =N-CH, 2.29-2.96 (m-4H,CH2),and 1.94, 1.92 (s, 6H, 2x. The 13C-NMR spectra for compound (6), shows the signals (400 MHz, DMSO-d6, δ in ppm): δ =172.453 (COOH) for 136.68 for N-C , 130.25 for NH-CH=C,109.78for (-C=C-), 118.78- 127.70 for Ar-C, 111.79 for O=C-C=C, 55.00 for CH3-C-CH3, 27.49 for aliphatic CH2, 21.60 for (2xCH3).

Anticancer Activity: Solutions used in this experiment (Culture Media RPMI-1640, Trypsin–Versin solution, Crystal Violet Stain and Trypan blue stain) were prepared according to Giuliano method.. The HepG2 Cell Line and normal cell Line (Rhabdomyosarcoma) (RD) was grown in RPMI-1640 medium equipped with 10% calf fetal serum according to the Freshney method.. The cytotoxicity assay was carried out using the crystal violate stain according to the method of Freshney (2012). The target compounds were dissolved in DMSO-d6 and diluted by serum free media (SFM) to prepare different concentrations range of (50,100) µg/ml. Two types of cell lines were used human liver cancer (HepG2), and normal human (RD)(RPMI-1640) cell lines. The tumor cells (1x105cell/ml) were seeded in 96-well microplate and incubated for 24 hours at 37C°, and then old media was changed with new serum-free medium (SFM) containing concentrations of each compound. Plate was incubated for 24 hours in humidified incubator at 37 C° containing 5% CO2. After incubation, the culture medium was discarded and 100 µl of

crystal violate was added to each, and re-incubated 20 min at 37 Cº. The results were read by using the ELISA with a wavelength of 492 nm. The inhibiting ratio was calculated according to the equation. Percentage of cell inhibition = (absorbance reading of control cells - absorbance reading of reated cells for each concentration/absorbance reading of control cells) x 100 NMR Data Analysis

NMR SPECTRA

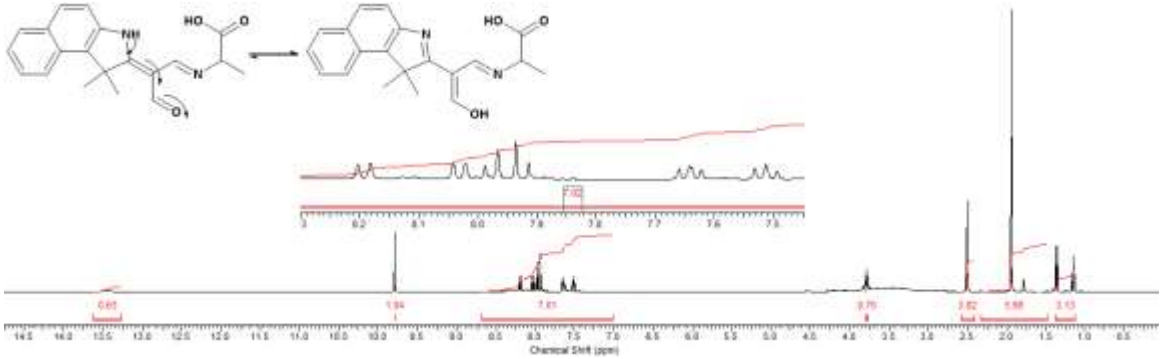

Figure 1: 1H NMR for Compound 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)propanoic acid (3)

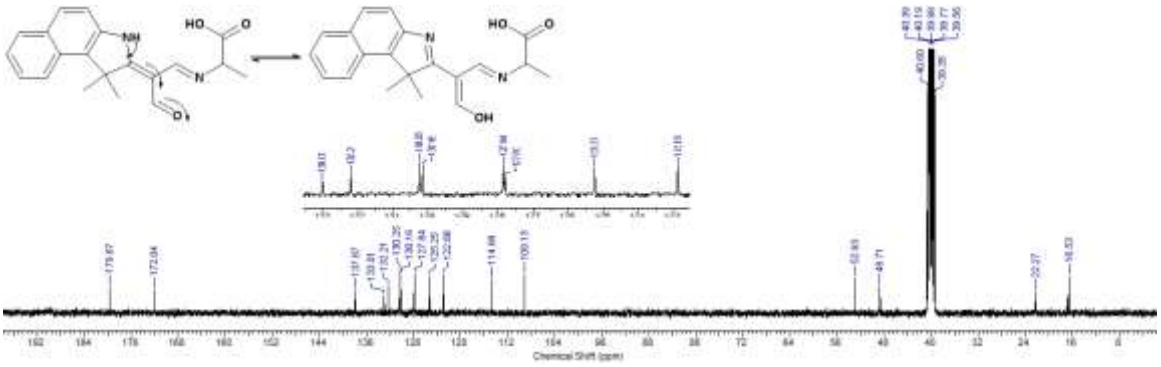

Figure 2: 13C NMR for Compound 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)propanoic acid (3)

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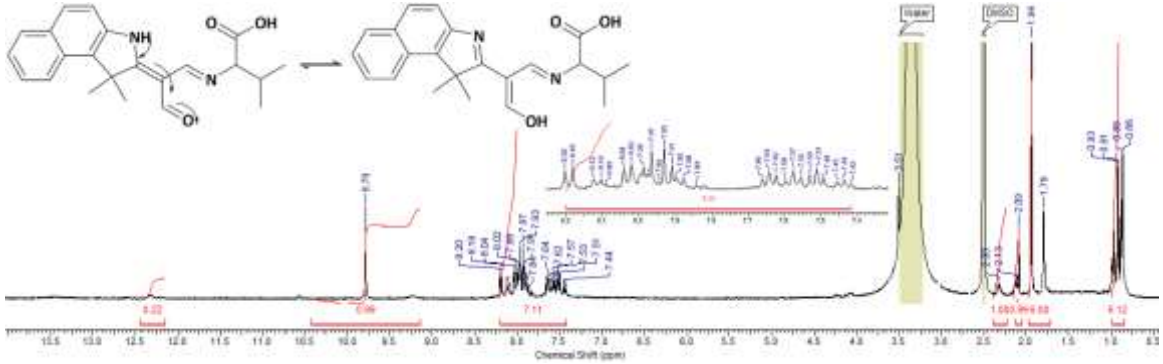

Figure 3: 1H NMR for Compound 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)-3-methylbutanoic acid (4)

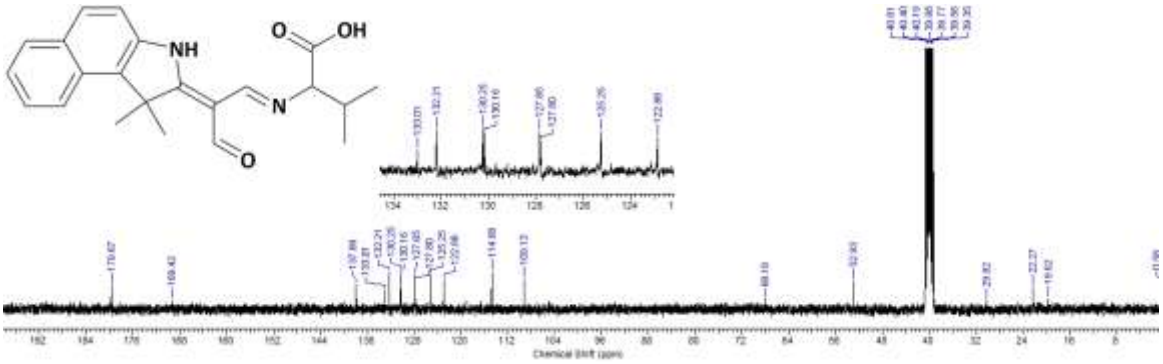

Figure 4: 13C NMR for Compound 2-((E)-((E)-2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)-3-oxopropylidene)amino)-3-methylbutanoic acid (4)

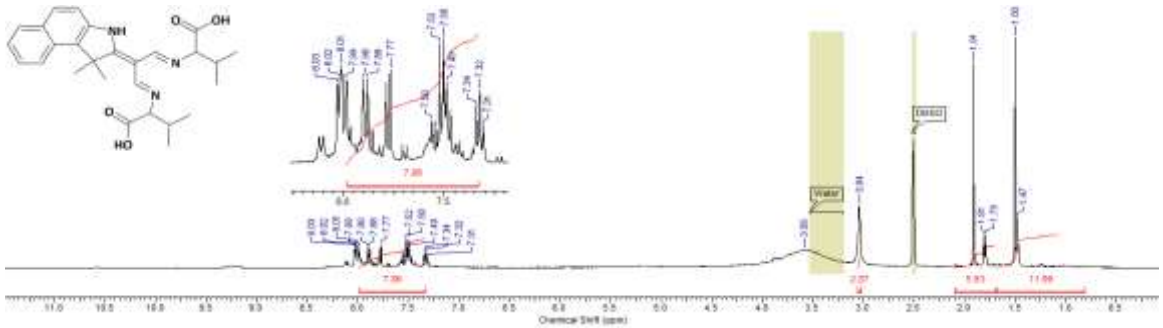

Figure 5: 1H NMR for Compound 2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)propane-1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-methylbutanoic acid) (5)

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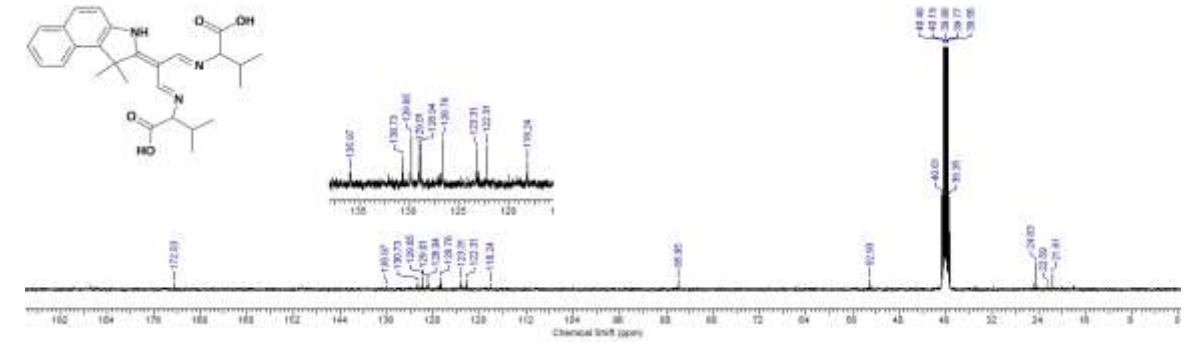

Figure 6: 13C NMR for Compound 2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)propane-1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-methylbutanoic acid) (5)

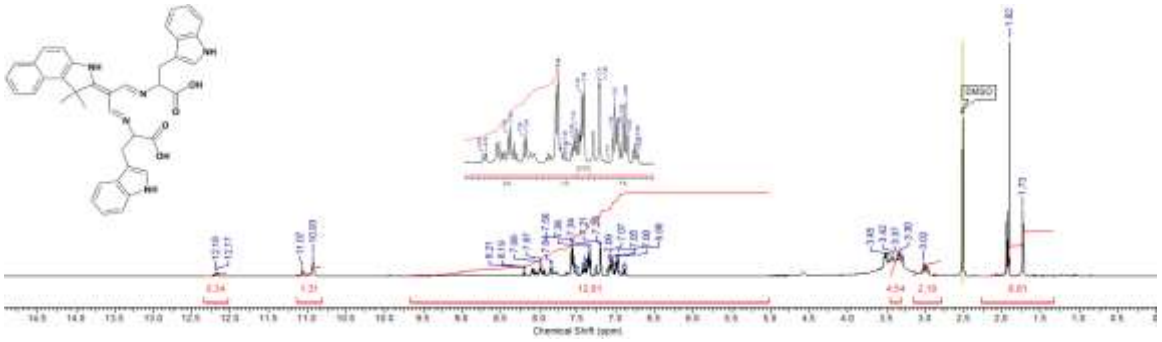

Figure 7: 1H NMR for Compound 2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)propane-1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-(1H-indol-3-yl)propanoic acid) (6)

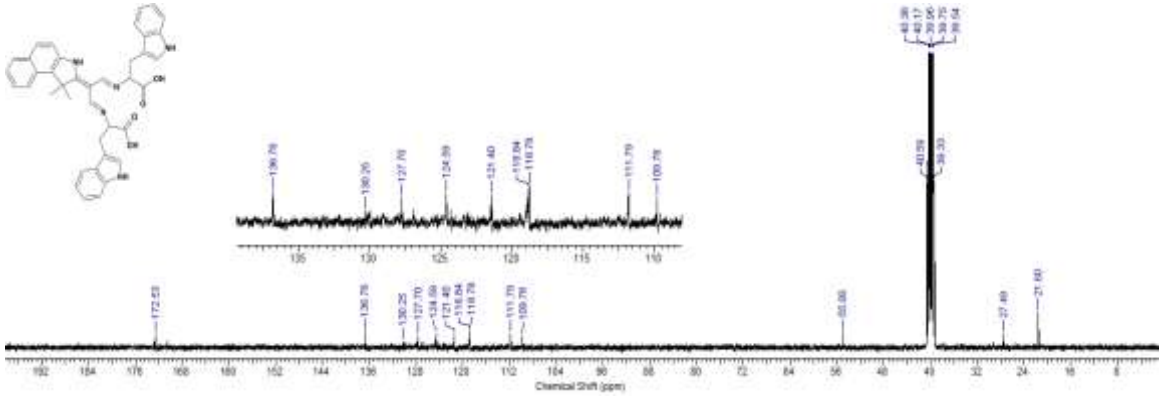

Figure 8: 13C NMR for Compound 2,2'-(1E,1'E)-(2-(1,1-dimethyl-1H-benzo[e]indol-2(3H)-ylidene)propane-1,3-diylidene)bis(azan-1-yl-1-ylidene)bis(3-(1H-indol-3-yl)propanoic acid) (6)

References

1. V. Sathya, A. Deepa, L. K. Sangeetha, V. Srinivasadesikan, S. L. Lee, V. Padmini, Development of Optical Biosensor for the Detection of Glutamine in Human Biofluids Using Merocyanine Dye. Journal of Fluorescence, 1-8(2022)
2. G. O. Menéndez, M. E. Pichel, C. C. Spagnuolo, E. A. Jares-Erijman, NIR fluorescent biotinylated cyanine dye: optical properties and combination with quantum dots as a potential sensing device. Photochemical & Photobiological Sciences, 12(2), 236-240(2013)
3. K. Zhang, J. Meng, W. Bao, M. Liu, X. Wang, Z. Tian, Mitochondrion-targeting near-infrared fluorescent probe for detecting intracellular nanomolar level hydrogen sulfide with high recognition rate. Analytical and Bioanalytical Chemistry, 413(4), 1215-1224(2021)
4. K. Miki, A. Kimura, K. Oride, Y. Kuramochi, H. Matsuoka, H. Harada, K. Ohe, High-Contrast Fluorescence Imaging of Tumors In Vivo Using Nanoparticles of Amphiphilic Brush-Like Copolymers Produced by ROMP. Angewandte Chemie International Edition, 50(29), 6567-6570(2011)
5. S. Onoe, T. Temma, K. Kanazaki, M. Ono, H. Saji, Development of photostabilized asymmetrical cyanine dyes for in vivo photoacoustic imaging of tumors. Journal of biomedical optics, 20(9), 096006(2015)
6. P. Arora, V. Arora, H. S. Lamba, D. Wadhwa, Importance of heterocyclic chemistry: a review. International Journal of Pharmaceutical Sciences and Research, 3(9), 2947(2012)
7. P. F. Lamie, W. A. Ali, V. Bazgier, L. Rárová, Novel N-substituted indole Schiff bases as dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase enzymes: synthesis, biological activities in vitro and docking study. European journal of medicinal chemistry, 123, 803-813(2016)
8. S. Kumar, D. N. Dhar, P. N. Saxena, Applications of metal complexes of Schiff bases. Journal of Scientific and Industrial Research, 68(03), 181-187(2009)
9. D. Sinha, A. K. Tiwari, S. Singh, G. Shukla, P. Mishra, H. Chandra, A. K. Mishra, Synthesis, characterization and biological activity of Schiff base analogues of indole-3-carboxaldehyde. European journal of medicinal chemistry, 43(1), 160-165(2008)
10. A. R. Saundane, K. N. Mathada, Synthesis, characterization, and biological evaluation of Schiff bases containing indole moiety and their derivatives. Monatshefte für Chemie-Chemical Monthly, 146(10), 1751-1761(2015)
11. EI. Grace, Synthesis, characterization and antimicrobial studies of Mn (II), Co (II), and Zn (II) Schiff base complexes derived from glycine and 2-hydroxy-1-napthaldehyde. International Journal of Innovation and Scientific Research, 18 (1), 1-3(2015)

12. AP. Rajput, PD. Girase, Review Article on Vilsmeier — Haack Reaction, Chem Inform, 44 (21), (2013)

13. M. Bollyn, Thermal Hazards of the Vilsmeier− Haack Reaction on N, N-Dimethylaniline. Organic process research & development, 9(6), 982-996(2005)

14. K. Seema, Yogeshstudy of vilsmeier – hack reagent. International journal for research publication & seminar. 2278-6848(2016)

15. S. M. Salih, H. M. Alkubaisi, F. L. Faraj, Synthesis and Characterization of Novel Pyrazole Derivatives from 4-Florophenylhydrazine and Study Their Cytotoxicity as Anti-Cancer Agent 11. Egyptian Journal of Chemistry, 64(11), 5-6(2021)

16. R. A. Mekheimer, M. A. Al-Sheikh, H. Y. Medrasi, K. U. Sadek, Advancements in the synthesis of fused tetracyclic quinoline derivatives. RSC advances, 10(34), 19867-19935(2020)

17. Z. M. Essam, G. E. Ozmen, D. Setiawan, R. R. Hamid, R. M. Abd El-Aal, R. Aneja, M. Henary, Donor acceptor fluorophores: synthesis, optical properties, TD-DFT and cytotoxicity studies. Organic & biomolecular chemistry, 19(8), 1835-1846(2021)

18. N. BERBER, Preparation and Characterization of Some Schiff Base Compounds. Adıyaman University Journal of Science, 10(1), 179-188(2020)

19. G. Calvaruso, M. Giuliano, P. Portanova, O. Pellerito, R. Vento, G. Tesoriere, Hsp72 controls bortezomib-induced HepG2 cell death via interaction with pro-apoptotic factors. Oncology reports, 18(2), 447-450(2007)

20. R. I. Freshney, Cytotoxicity. Culture of animal cells: a manual of basic technique, (2005)

21. S. S. a. M. L. Larramendy, ed. Introductory Chapter: Cytotoxicity, 2021)

22. R. I. Freshney, Culture of animal cells: a manual of basic technique and specialized applications,(John Wiley & Sons, 2015)