

Synthesis, characterization, molecular docking, in silico ADME study, and in vitro cytotoxicity evaluation of new pyridine derivatives of nabumetone.

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Abstract:

New pyridine derivatives of nabumetone containing 2-amino 3-cyano moieties were synthesized and aimed to introduce new EGFR kinase inhibitors through two methods either by synthesis of chalcone derivatives initially (1a-d)

followed by reacting it with malononitrile and ammonium acetate to form (2a-d) or from a one-pot synthesis of all reactants together to synthesis compounds (2a-e). Melting point, and FT-IR spectra were used to characterize all the synthesized compounds and were confirmed by ¹H-NMR, and ¹³C-NMR spectroscopy. The final compounds (2a-e) were investigated in vitro against A549 (lung cancer cell line) and WRL68 (human normal cell line). compounds (2a, 2b, and 2e) produced marked cytotoxic activity with IC₅₀ (24.62, 23.43, and 24.06 µg/ml) respectively, higher than what obtained from erlotinib with IC₅₀ (25 µg/ml) as a reference drug. Measuring the selectivity index (SI) reveals that all the compounds have high selectivity especially compound (2a) being the most selective towards cancerous cells rather than normal cells with SI two folds higher than erlotinib. The molecular docking study reveals good binding to the EGFR kinase that has a good correlation to the MTT Assay results. In silico ADME study exposes that this synthesized series not only have interesting activity but also shows promised pharmacokinetic properties.

Key words: Nabumetone, EGFR inhibitor, cyanopyridine, anticancer, molecular docking.

تصنيع والتشخيص والأرساء الجزيئي و دراسة الخواص (الامتصاص والتمثيل الغذائي والتوزيع والأفراز) و التقييم المختبري للفعالية المضادة للأورام لمشتقات البيريدين الحديثة للنابيوميتون

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الخلاصة:

تم تصنيع مجموعه جديدة من مشتقات البيريدين للنابيوميتون و التي تحتوي على مجموعة ٢-امين و ٣-سيانيد, هذه الدراسة تهدف الى تقديم مجموعه جديدة من مثبطات البروتين EGFR حيث تم تصنيع هذه المركبات بطريقتين اما عن طريق تصنيع مشتقات الجالكون (a-d¹) اولاً متبوعاً بـتفاعل مع مالونونتريل و الأمونيوم أسيتيت لتصنيع (a-d²) المركبات النهائية أو من خلال تخليق وعاء واحد لجميع المواد المتفاعلة معاً لإنتاج (a-e²). أن جميع المركبات المصنعة تم تشخيصها عن طريق درجة الانصهار, ¹H-NMR, FT-IR, و ¹³C-NMR. تم فحص المركبات النهائية (a-e²) في

المختبر ضد خلايا سرطان الرئة A549 و خلايا جسم الانسان الطبيعية WRL68 . كان للمركبات على وجه التحديد (a, 2b& 2e) والتي أنتجت نشاطاً ساماً للخلايا بشكل ملحوظ بقيم IC50 (٢٤,٦٢ و ٢٣,٤٣ و ٢٤,٠٦ مايكروغرام/مل) على التوالي والتي حققت قيماً أعلى مقارنة مع عقار الألوكتينيب كدواء مرجعي و الذي يحتوي على قيمة IC50 (٢٥ مايكروغرام/مل). قياس مؤشر الانتقائية SI كشف ان جميع المركبات لديها هوامش سلامة عالية تجاه الخلايا الطبيعية وأنقائية عالية تجاه الخلايا السرطانية وعلى وجه التحديد المركب a٢ بأنقائية أعلى بمرتين من عقار الألوكتينيب. دراسة الألتحام الجزيئي كشف عن ارتباط مثالي بلبروتين EGFR والتي أظهرت توافقاً مع النتائج المختبرية للفعالية الدوائية. كما ان دراسة الخواص (الامتصاص والتمثيل الغذائي والتوزيع والأفراز) كشف ان هذه السلسلة من المركبات ليس لها نشاط سرطاني مثير للاهتمام فحسب , بل تظهر ايضاً خصائص حركية دوائية موعودة.

الكلمات المفتاحية: النابيوميتون, مثبطات ال EGFR, سيانيد البيريدين, مضاد للسرطان, الأرساء الجزيئي.

Introduction

Cancer is the major leading cause of death worldwide. Its accounts for one death out of every six. This number indicates a real crisis in worldwide public health and healthcare systems. On the top of the list is "lung cancer."^[1]

This is an urgent medical need to discover and innovate new approaches to control cancer global issues. One of the challenges that scientists face in developing highly effective and selective anti-cancer drugs is chemoresistance; a higher dose of treatment is required for that, which leads to greater toxicity in cancer patients ^[2]. Therefore, researchers nowadays focused on developing novel anticancer drugs to fulfill this purpose. The epidermal growth factor receptor (EGFR), which regulates different cellular functions, is one of the membrane proteins that has received substantial study, this protein has also been found to be mutated in lung cancer ^[3].

Heterocyclic rings especially those that are nitrogen-based, are among the most important in chemistry because of their diverse biological activities. particularly Amino pyridine scaffolds have many biological activities and have been incorporated in many anticancer drugs, examples of drugs approved by the food and drug administration (FDA) containing this moiety are illustrated in figure (1): the three TKIs crizotinib for the treatment of non-small cell lung carcinoma, bosutinib for myelogenous leukemia and pexidartinib for tenosynovial giant cell tumor. Niflumic acid, tenoxicam, and lornoxicam as anti-inflammatory

analgesics, Lasmiditan an anti-migraine drug, imiquimod for the treatment of genital wart and basal cell carcinoma, Dabigatran an anti-diabetic drug and Sulfapyridine an antibacterial agent ^[4-8]. A literature review has shown that the high therapeutic properties of amino cyanopyridine have motivated researchers over years to synthesize a wide number of 2-amino 3-cyanopyridine derivatives such as anticancer ^[9-11], antimicrobial ^[12,13], anti-inflammatory^[14].

According to our literature review and the above-mentioned findings, we had an interest in synthesizing novel cytotoxic agents directed to EGFR as inhibitors through modifying nabumetone a known (NSAID) by incorporating amino cyanopyridine moiety, moreover enhancing the cytotoxic activity through the incorporation of varied lipophilic and hydrophilic aromatic moieties. On the other hand, the pyridine ring enhances the hydrogen bond acceptor and donor with the kinase region, where the naphthyl group of the nabumetone confers a good binding to the receptor and enhance the pharmacokinetics properties.

All the new synthesized compounds were screened *in vitro* against the lung cancer line and normal cell line to investigate their affinity to EGFR followed by exploring the binding interaction of these molecules with the targeted receptor by molecular docking and *in silico* ADME study to predict and study the pharmacokinetic properties.

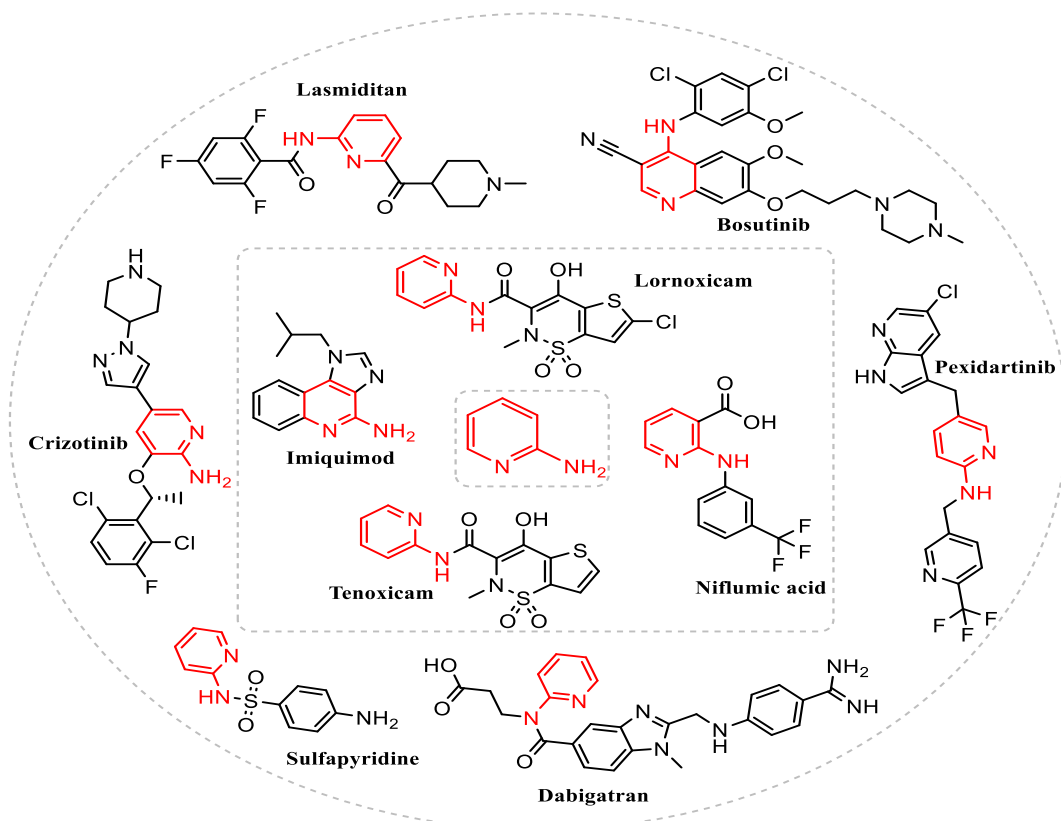


Figure (1): FDA approved containing amino cyanopyridine moiety.

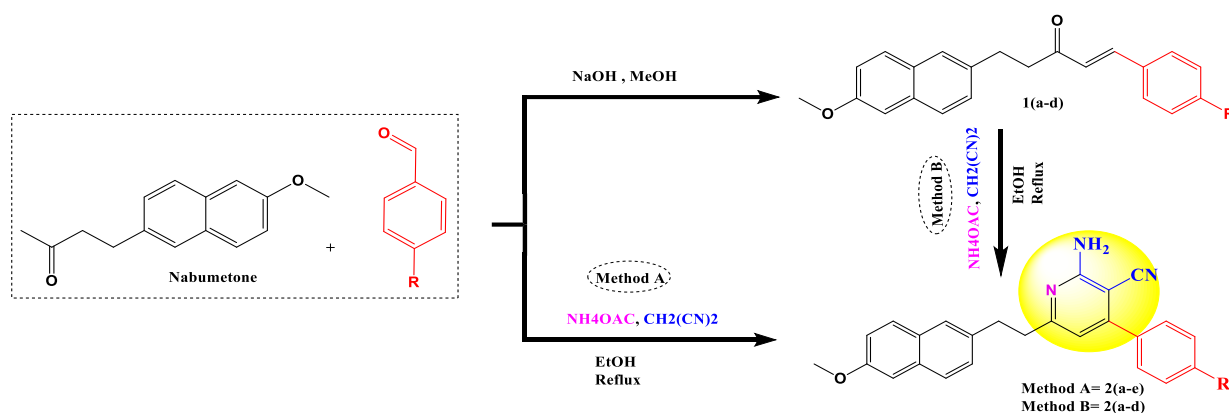
Experimental:

chemistry:

All the chemicals used in the preparation of the series compounds were purchased from Merck, Sigma Aldrich, Fluka, and Hyperchem. Melting points were assessed by an open capillary method using the Stuart melting point instrument. The IR spectra were measured by the Bruker

instrument. $^1\text{H-NMR}$ plus $^{13}\text{C-NMR}$ were obtained with Bruker 300 MHz spectrophotometer. TLC was used to observe the reaction on silica gel (60) F254 Merck aluminum sheet, the spots were revealed by exposure to UV light.

The general pathway that was adopted for the synthesis of new compounds and their intermediates is illustrated in scheme (1).



Scheme (1): Synthesis of 2,3,4,6 substituted derivative of pyridine.

where **R** (a-e) = CH_3 , OCH_3 , Cl , NO_2 , OH , respectively.

General synthesis procedure for the of intermediates 1(a-d):

In a round flask, nabumetone (0.456g, 2 mmol), and one of the 4-substituted benzaldehydes (a-d) (2 mmol) dissolved in 15ml absolute methanol followed by the addition of freshly prepared alcoholic NaOH solution (0.08gm, 2mmol in methanol: D.W. (5:2.5ml) solvent system. The mixture was then stirred for about 24 hr. at room temperature. Finally, the precipitate was then filtered, washed with distilled water until the filtrate neutralize litmus paper followed by recrystallization from methanol (15).

5-(6-methoxynaphthalen-2-yl)-1-(p-tolyl) pent-1-en-3-one 1a

MP: (130-132°C), white powder, 91% of yield, FT-IR cm^{-1} : 3048 (CH of aromatic), 2932 (CH of aliphatic), 1649 (C=O), 1606 (C=C adjacent to C=O), 1597 (C=C of aromatic), 1263 (methoxy group). $^1\text{H-NMR}$ (DMSO)(300 MHz): δ ppm 2.36 (singlet, 3H, CH_3), 3.04 (triplet, 2H, CH_2), 3.14 (triplet, 2H, CH_2), 3.88 (singlet, 3H, OCH_3), 6.90 (doublet, 1H, CH α to C=O), 7.15-7.43 (multiplet, 5H, Ar-H), 7.63 (multiplet, 3H, CH β to C=O and Ar-H), 7.68-7.78 (multiplet, 3H, Ar-H). $^{13}\text{C-NMR}$ (DMSO) (75 MHz) δ ppm 21.53, 30.09, 41.74, 55.59, 106.22, 118.99, 125.98, 126.53, 127.15, 128.28, 129.05, 129.27, 130.06, 132.21, 133.27, 136.90, 140.93, 142.73, 157.26, 199.63.

5-(6-methoxynaphthalen-2-yl)-1-(4-methoxyphenyl) pent-1-en-3-one 1b

MP:(126-128°C), milky powder, 89% of yield, FT-IR cm^{-1} : 3034 (CH of aromatic), 2962 (CH of aliphatic), 1636 (C=O), 1600 (C=C adjacent to C=O), 1572 (C=C of aromatic), 1246 (methoxy group); $^1\text{H-NMR}$ (DMSO)(300 MHz): δ PPM 3.04 (triplet, 2H, CH_2), 3.09 (triplet, 2H, CH_2), 3.81,3.87 (singlet, 3H, 3H, OCH_3), 6.81 (doublet, 1H, CH α to C=O), 7.00-7.41 (multiplet, 4H, Ar-H), 7.61 (doublet, 1H, CH β to C=O), 7.68 – 7.76 (multiplet, 5H, Ar-H). ^{13}C NMR (DMSO) (75 MHz) δ ppm 30.09, 41.74, 55.56, 55.78, 106.19,

114.89, 118.97, 124.62, 126.50, 127.15, 127.46, 128.27, 129.04, 129.26, 130.71, 133.25, 136.94, 142.62, 157.24, 161.63, 199.44.

1-(4-chlorophenyl)-5-(6-methoxynaphthalen-2-yl) pent-1-en-3-one 1c

MP: (128-130°C), off-white crystals, 85% of yield, FT-IR cm^{-1} : 3050 (CH of aromatic), 2964 (CH of aliphatic), 1683 (C=O), 1607 (C=C α to C=O), 1587 (C=C of aromatic), 1247 (methoxy group), 769 (C-Cl). $^1\text{H-NMR}$ (DMSO)(300 MHz): δ ppm 3.04 (triplet, 2H, CH_2), 3.12 (triplet, 2H, CH_2), 3.86 (singlet, 3H, CH_3), 6.95 (doublet, 1H, CH α to C=O), 7.14-7.50 (multiplet, 5H, Ar-H), 7.63 (doublet, 1H, CH β to C=O), 7.68 – 7.76 (multiplet, 5H, Ar-H). $^{13}\text{C-NMR}$ (DMSO)(75MHz) δ 29.92, 41.97, 55.57, 106.20, 118.97, 126.50, 127.16, 127.56, 128.25, 129.03, 129.46, 130.58, 133.26, 133.94, 135.38, 136.80, 141.24, 157.25,199.44.

5-(6-methoxynaphthalen-2-yl)-1-(4-nitrophenyl) pent-1-en-3-one 1d

MP: (135-137°C), brown powder, 72% of yield, FT-IR cm^{-1} : 3052 (CH of aromatic), 2955 (CH of aliphatic), 1641 (C=O), 1603 (C=C α to C=O), 1580 (C=C of aromatic), 1518,1390 (NO_2), 1265 (methoxy group); $^1\text{H-NMR}$ (DMSO)(300 MHz): δ ppm 3.04 (triplet, 2H, CH_2), 3.14 (triplet, 2H, CH_2), 3.87 (singlet, 3H, CH_3), 6.71 (doublet, 1H, CH α to C=O), 7.15-7.40 (multiplet, 3H, Ar-H), 7.63 (doublet, 1H, CH β to C=O), 7.68 – 7.77 (multiplet, 3H, Ar-H), 8.02 (doublet, 2H, Ar-H), 8.20 (doublet, 2H, Ar-H).

General synthesis procedure for the 2-amino 3-cyanopyridine series (2a-e):

Method A: a solution of each of Nabumetone (0.228g, 1 mmol), appropriate aromatic benzaldehyde (a-e) (1mmol), Ammonium acetate (0.616g, 8mmol), and Malononitrile (0.528g, 8mmol) in (15ml) absolute Ethanol were allowed to heat under reflux in an oil bath for (3 - 5 hr.).

Method B: a solution of chosen compound (1a-d) (1mmol), Ammonium acetate (0.616g, 8mmol), and Malononitrile (0.528g, 8mmol) were added in (12ml) absolute Ethanol and allowed to heat under reflux in an oil bath for (3 - 5 hr.).

For both methods, the reaction has been monitored by TLC. A precipitate was formed, it was filtered off while the reaction mixture was still hot, followed by washing the precipitate with iced distilled water and acetone to afford the corresponding compounds (16).

2-amino-6-(2-(6-methoxynaphthalen-2-yl) ethyl)-4-(p-tolyl) nicotinonitrile (2a)

MP:(188-189°C), Beige powder, 85% (method A) and 86% (method B) of yield, FT-IR cm^{-1} : 3478, 3354 (NH_2), 3047 (CH of aromatic), 2958 (CH of aliphatic), 2212 ($\text{C}\equiv\text{N}$), 1608 ($\text{C}=\text{N}$ of pyridine), 1579 ($\text{C}=\text{C}$ of aromatic), 1263 (methoxy group); $^1\text{H-NMR}$ (DMSO)(300 MHz): δ ppm 2.38 (singlet, 3H, CH_3), 3.04 (triplet, 2H, CH_2), 3.10 (triplet, 2H, CH_2), 3.88 (singlet, 3H, OCH_3), 6.69 (singlet, 2H, NH_2), 7.15-7.80 (multiplet, 11H, Ar-H). $^{13}\text{C-NMR}$ (DMSO) (75 MHz) δ ppm 21.30, 36.09, 37.07, 55.61, 85.82, 95.47, 106.25, 116.69, 119.09, 126.76, 127.32, 128.05, 128.74, 128.99, 129.33, 129.64, 133.42, 135.21, 135.98, 139.47, 151.79, 154.11, 157.39, 161.91.

2-amino-6-(2-(6-methoxynaphthalen-2-yl)ethyl)-4-(4-methoxyphenyl) nicotinonitrile (2b)

MP:(174-179°C), Light yellow powder, 85% (method A) and 86% (method B) of yield, FT-IR cm^{-1} : 3434, 3351 (NH_2), 3054 (CH of aromatic), 2934 (CH of aliphatic), 2210 ($\text{C}\equiv\text{N}$), 1606 ($\text{C}=\text{N}$ of pyridine), 1569 ($\text{C}=\text{C}$ of aromatic), 1269 (methoxy group); $^1\text{H-NMR}$ (DMSO)(300 MHz): δ ppm 3.04 (triplet, 2H, CH_2), 3.09 (triplet, 2H, CH_2), 3.82, 3.88 (singlet, 3H, OCH_3), 6.69 (singlet, 2H, NH_2), 7.05-7.80 (multiplet, 11H, Ar-H). $^{13}\text{C-NMR}$ (DMSO)(75 MHz) δ ppm 36.09, 37.07, 55.61, 55.78, 81.82, 95.13, 106.24, 114.50, 116.92, 119.09, 126.75, 127.32, 128.06,

128.99, 129.33, 130.20, 130.32, 133.41, 136.01, 151.69, 154.16, 157.39, 160.60, 162.02.

2-amino-4-(4-chlorophenyl)-6-(2-(6-methoxynaphthalen-2-yl)ethyl) nicotinonitrile (2c)

MP:(210-213°C), light yellow powder, 83% (method A) and 85% (method B) of yield, FT-IR cm^{-1} : 3463, 3348 (NH_2), 3051 (CH of aromatic), 2963 (CH of aliphatic), 2215 ($\text{C}\equiv\text{N}$), 1609 ($\text{C}=\text{N}$ of pyridine), 1577 ($\text{C}=\text{C}$ of aromatic), 1264 (methoxy group), 772 (C-Cl); $^1\text{H-NMR}$ (DMSO)(300 MHz): δ ppm 2.90 (triplet, 2H, CH_2), 3.08 (triplet, 2H, CH_2), 3.88 (singlet, 3H, OCH_3), 6.73 (singlet, 2H, NH_2), 7.14-7.80 (multiplet, 11H, Ar-H). $^{13}\text{C-NMR}$ (DMSO) (75 MHz) δ ppm 36.08, 37.10, 55.61, 81.54, 96.05, 106.24, 116.45, 119.09, 126.75, 127.33, 128.05, 128.98, 129.12, 129.34, 130.75, 133.42, 134.70, 135.96, 136.90, 152.09, 154.05, 157.39, 162.20.

2-amino-6-(2-(6-methoxynaphthalen-2-yl)ethyl)-4-(4-nitrophenyl) nicotinonitrile (2d)

MP:(220-222°C), light yellow powder, 74% (method A) and 72% (method B) of yield, FT-IR cm^{-1} : 3466, 3355 (NH_2), 3008 (CH of aromatic), 2966 (CH of aliphatic), 2210 ($\text{C}\equiv\text{N}$), 1601 ($\text{C}=\text{N}$ of pyridine), 1579 ($\text{C}=\text{C}$ of aromatic), 1518, 1392 (NO_2), 1257 (methoxy group); $^1\text{H-NMR}$ (DMSO)(300 MHz): δ ppm 2.99 (triplet, 2H, CH_2), 3.09 (triplet, 2H, CH_2), 3.87 (singlet, 3H, OCH_3), 6.76 (singlet, 2H, NH_2), 7.13-7.80 (multiplet, 9H, Ar-H), 8.33 (doublet, 2H, Ar-H); $^{13}\text{C-NMR}$ (DMSO)(75 MHz) δ ppm 36.05, 37.12, 55.60, 87.21, 96.85, 106.23, 116.18, 119.10, 124.11, 126.75, 127.35, 128.03, 128.97, 129.33, 130.41, 133.42, 135.90, 144.38, 147.64, 152.32, 154.01, 157.40, 162.31.

2-amino-4-(4-hydroxyphenyl)-6-(2-(6-methoxynaphthalen-2-yl)ethyl) nicotinonitrile (2e)

MP:(218-220°C), mustard yellow powder, 83% (method A) of yield, FT-IR cm^{-1} :

3406, 3328 (NH₂), 3001 (CH of aromatic), 2936 (CH of aliphatic), 2213 (C≡N), 1609 (C=N of pyridine), 1572 (C=C of aromatic), 1269 (methoxy group); ¹H-NMR (DMSO)(300 MHz): δ ppm 2.95 (triplet, 2H, CH₂), 3.10 (triplet, 2H, CH₂), 3.88 (singlet, 3H, OCH₃), 6.65 (singlet, 2H, NH₂), 6.87-7.80 (multiplet, 9H, Ar-H), 7.86 (doublet, 2H, Ar-H); ¹³C-NMR (DMSO)(75 MHz) δ ppm 36.09, 37.07, 55.60, 85.83, 97.24, 107.70, 116.78, 119.22, 126.65, 127.34, 128.08, 129.06, 129.37, 130.07, 133.42, 134.09, 136.16, 151.79, 154.79, 158.65, 159.35, 162.01.

Cytotoxic activity

Anticancer activity of the final compounds (2a-e) which we synthesized were tested by MTT Assay which was performed according to Freshney method (17). The cytotoxic activity was studied *in vitro* against lung cancer cell line (A549) and normal cells (WRL-68) to assess their selectivity towards EGFR. Erlotinib was employed as a standard drug for cytotoxic activity. The cells were obtained from College of Biotechnology, Al-Nahrain University. Cancer cells (1x10⁴ to 1x10⁶ cells/ml) were allowed to grow in 96 flats well plates, proceeded by incubation at 37 °C, and 5 % CO₂ for 24 hrs. followed by removing the medium and 2-fold serial dilution of the desired compounds concentrations ranging from (25-400 µg/mL) were added for 24 hrs. MTT solution about (10 µl) was added, then Plates were again incubated for a further 4 hrs. After incubation, the media were cautiously removed followed by adding 100 µL of solubilization solution for 5 min. By using an ELISA reader for measurement at 575 nm wavelength. The optical density data was applied to statistical analysis by Prism pad software to evaluate the IC₅₀.

Molecular docking

The molecular docking studies for the new synthesized molecules (2a-e) were performed using The CCDC GOLD

(Cambridge Crystallographic Data Center) (version 5.6.2)(18). Hermes visualizer program (version1.9.2) is used to visualize receptors, ligands, interactions (H bonds, short contact), and the active site (19,20).

Ligand and protein receptor preparation.

The crystal structure of the EGFR was taken from the Protein Data Bank (PDB ID:4HJO)(21,22), ChemDraw (version 19.1) was employed for drawing all the ligands structures, then convert them to the 3-D structures by Chem3D (version 19.1) and to minimize their energies by MM2 force. H atoms were added to the ligand-receptor complex and H₂O molecules were removed(23).

Molecular docking approach

The docking process is initiated by putting up the receptors in Hermes Structure visualization software. Chemscore kinase was utilized as a configuration template and CHEMPLP as a scoring function and for recording all the solutions by its fitness function. The molecules' interaction with the residue of the EGFR was checked using docking outcomes which were the docked posture, binding mode, and the free energy of binding.

ADME and physiochemical properties

The SwissADME server was utilized to evaluate the physicochemical and pharmacokinetic properties of 2(a-e). ChemDraw (V. 19.1) was utilized to generate the chemical structure of the intended molecules, which was then transformed to SMILE to complete the ADME prediction in the webserver (24,25).

Results and discussion

Chemistry

Initially, the ketone represented as nabumetone reacts with the appropriate aromatic benzaldehydes (a-d) catalyzed by a base in an aldol condensation to form chalcone series (1a-d) and then cyclocondensation with Ammonium

acetate and Malononitrile to form (2a-d). furthermore, the same final compounds (2a-e) were prepared by mixing all the reactants (Nabumetone, one of the 4-substituted benzaldehydes, malononitrile and ammonium acetate) in a suitable amount of solvent. The reaction progress for the two methods was monitored by TLC. The intermediates (1a-d) and final compounds (2a-e) were identified and confirmed via their FT-IR, ^1H -NMR, and ^{13}C -NMR spectra data. for example, the H-NMR data for compound (1a) showed two doublet bands for the α and β vinylic protons at (6.90) and (7.63) respectively. The IR data for (2a) showed a distinctive band at (3478, 3354) cm^{-1} and (2212) cm^{-1} for the NH_2 and $\text{C}\equiv\text{N}$ respectively, and the H-NMR for (2a) showed a singlet peak

related to NH_2 protons at (6.69) ppm; the C-NMR showed a new peak for $\text{C}\equiv\text{N}$ at (116.69) ppm.

Cytotoxicity activity

Compounds (2a-e) that we synthesized were studied to determine their cytotoxic activities in *vitro* on lung cancer cell line (A549) by MTT Assay and using Erlotinib as a reference drug at five different concentrations (25, 50, 100, 200, 400 $\mu\text{g/mL}$). the cells treated with the compounds to get the IC_{50} values. The compounds were also tested against normal cells (WRL-68) to determine the new compound's selectivity toward cancer cells and their safety. The IC_{50} and the selectivity index (SI) values were outlined in table (1).

Table (1): the IC_{50} of the new tested compounds (2a-e) and erlotinib as a positive control with their SI.

Comp.	IC_{50} $\mu\text{g/ml}$ (A549)	IC_{50} $\mu\text{g/ml}$ (WRL68)	SI
2a	24.62	411.9	16.73
2b	23.43	120.2	5.13
2c	70.6	209.9	2.97
2d	79.4	355.5	4.47
2e	24.06	148.9	6.18
Erlotinib	25.08	214.5	8.55

According to the mentioned values, the newly synthesized compounds (2a, 2b, 2e) showed comparable anticancer activity. The most remarkable cytotoxic effect was noted for compound (2b) with an IC_{50} of 23.43 ($\mu\text{g/ml}$), making it as effective as the standard medication erlotinib, with an IC_{50} of 25.08 ($\mu\text{g/ml}$). While compounds (2c and 2d) have higher IC_{50} (70.6 and 79.4 $\mu\text{g/ml}$) than Erlotinib, which means that they possess lower anticancer activity

than it. The synthesized compounds are proven to have little effect on normal cells as the concentration required for the cytotoxicity activity on normal cells (WRL68) (120.2-411.9 $\mu\text{g/ml}$) are much higher than those required on cancerous cells (A549) (23.4-79.4 $\mu\text{g/ml}$). This indicates that those molecules are safe on non-cancerous cells as notable by their IC_{50} , the compound recognizes to be highly selective when the $\text{SI} \geq 3$ (26).

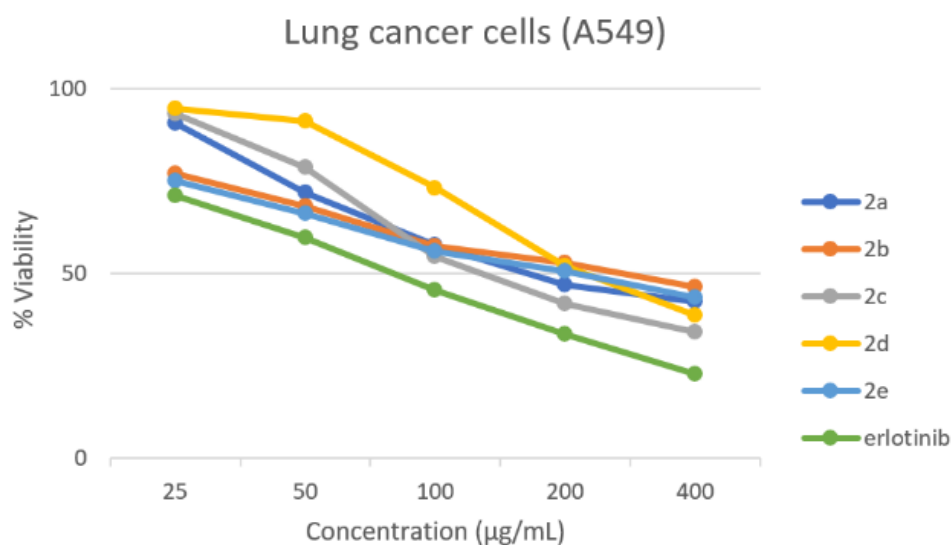


Figure (2): viability% curve of compou-nds (2a-e) and erlotinib in lung cancer cell line (A549) after treating by (25-400 µg/mL).

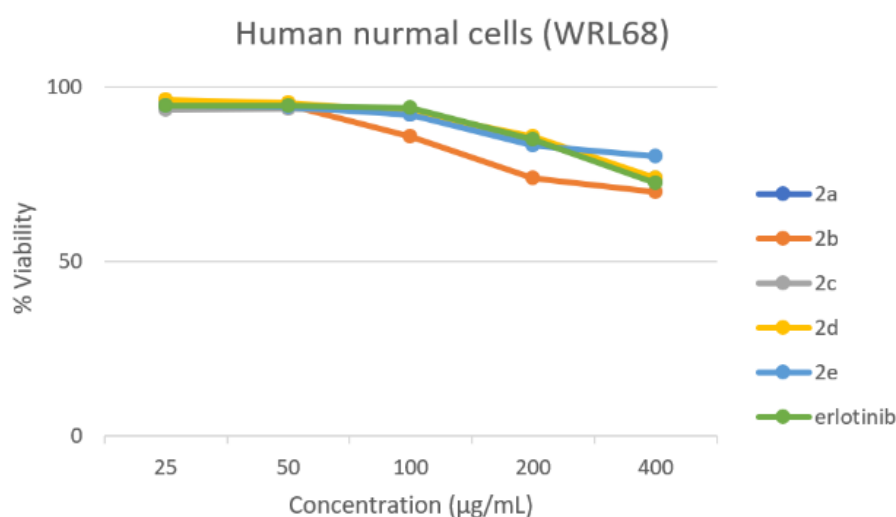


Figure (3): viability% curve of compou-nds (2a-e) and erlotinib in human normal cell line (WRL68) after treating by (25-400 µg/mL).

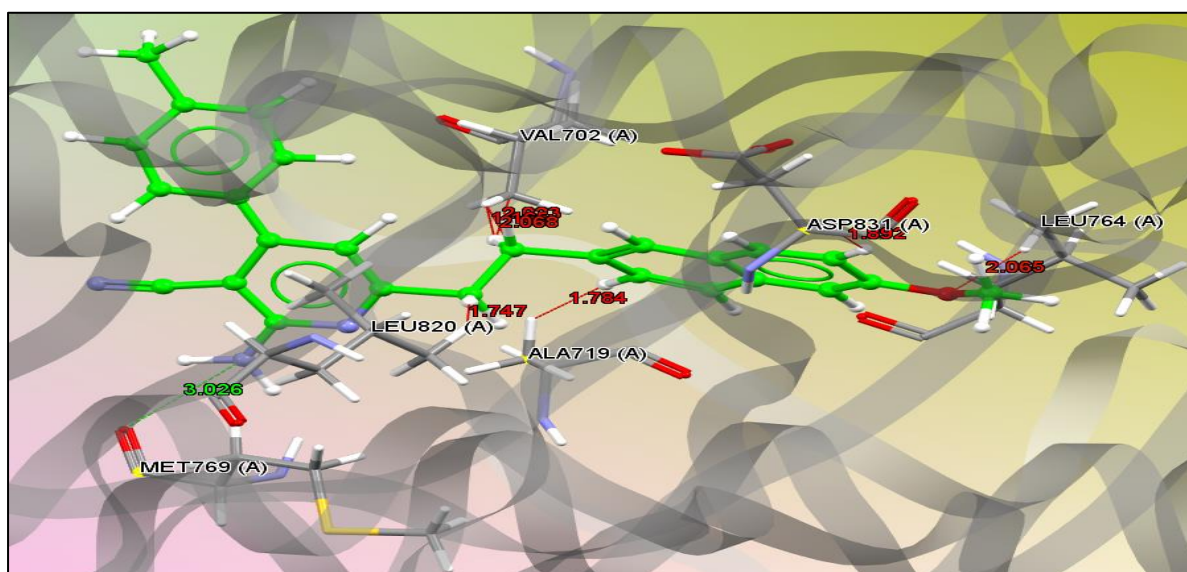
Molecular docking

the docking study of the new compounds and the standard drug erlotinib results were shown in table (2) and figures (4, 5, 6, and 7). Docking results illustrate that all the docked compounds (2a-e) have binding energies comparable to that of erlotinib in the proteins' active site, suggesting a potential interaction with the EGFR protein,

as it binds through H bonding in addition to other short contacts that improve the binding. the docking results for compound (2a) revealed that it forms a hydrogen bond with MET 769 via NH₂ moiety and 7 short contacts with different amino acids (4 bonds through the ethylene bridge, 2 bonds through the naphthyl group, 1 bond with the methoxy group).

Table (2): The free binding energies of the final derivatives and erlotinib in EGFR.

Compound	EGFR Binding Energy (PLPfitness)	Amino Acids Involved in H- bonding	Amino Acids involved in short contact Interactions
2a	86.5	MET 769	LEU 764, ASP 831, LEU 820, ALA 719, VAL 702 (3)
2b	87.3	-	LEU 768, LEU 694, LEU 820, VAL 702, LEU 764.
2c	84.25	MET 769, 2 HOH bridge with THR 766 & THR 830.	MET 769, LEU 694 (4), LYS 721 (3), VAL 702 (5), THR 766, ASP 831 (2), MET 742, LEU 753
2d	76.99	MET 769	CYS 773 (2), LEU 768, LEU 834, ASP 831, THR 766, ALA 719, VAL 702 (4), LEU 697 (3)
2e	86.14	-	LEU764, LEU 820, LEU 694 (2), LYS 704, LEU 768
Erlotinib	85.44	LYS 704	CYS 773, GLY 695, HOH bridge with THR 830 & THR 766.

**Figure (4): The orientation and the binding interaction of compound (2a) inside EGFR**

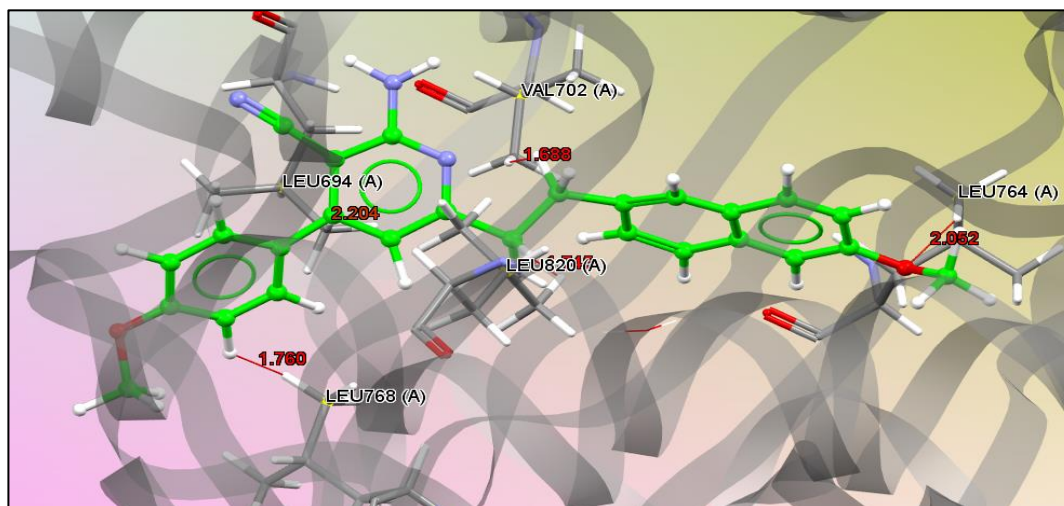


Figure (5): The orientation and the binding interaction of compound (2b) inside the EGFR.

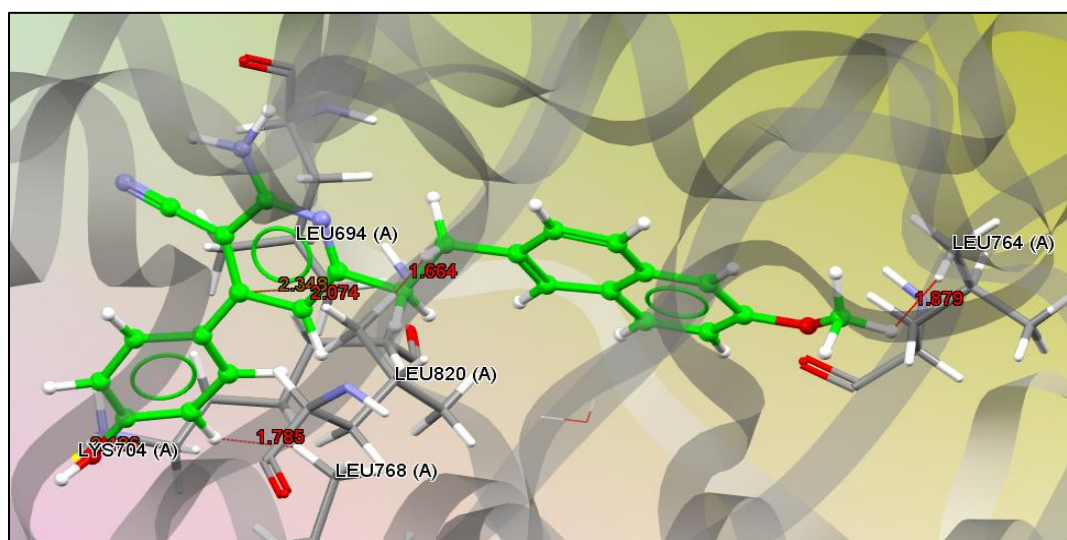


Figure (6): The orientation and the binding interaction of compound (2e) inside the EGFR.

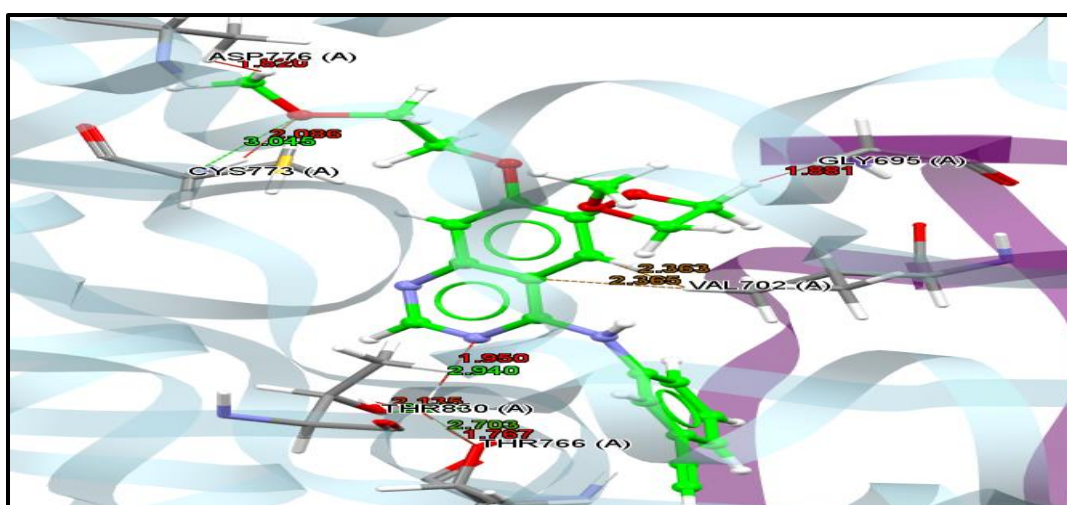


Figure (7): The orientation and the binding interaction of erlotinib inside the EGFR.

ADME and physiochemical properties

The pharmacokinetic properties of the synthesized molecules were analyzed (absorption, distribution, metabolism, and excretion), The drug-like features of all molecules were investigated as shown in table (3). Lipinski's rule of five, this rule is extensively devoted as a filter for molecules that will probably be utilized as a lead for drug innovation design. In a brief, Lipinski's rule of five relates to the orally taken drugs that must contain the following features to be able to be administered

orally: (1) ≤ 5 hydrogen bonds donor (2) ≤ 10 hydrogen bond acceptor (3) $MlogP \leq 5$ (4) molecular weight ≤ 500 . The topological polar surface area (TPSA) was also measured, as this is a crucial characteristic of drug bioavailability (27). All the compounds obey the Lipinski rule with no number of violations, all have TPSA less than 140 \AA^2 ranging from (71-117 \AA^2) with high gastrointestinal absorption and no permeation through blood brain barrier so these molecules are expected to not cause any CNS side effects.

Table (3): ADME results of the targeted new compounds

Comp	H-donor	H-acceptor	MW	Log Po/w (MLOGP)	Lipinski violation	TPSA	Rotatable bonds	GI abs	BBB permeate
2a	1	3	393.4	3.47	Yes	71.93	5	High	No
2b	1	4	409.4	2.91	Yes	81.16	6	High	No
2c	1	3	413.9	3.74	Yes	71.93	5	High	No
2d	1	5	424.4	2.31	Yes	117.75	6	low	No
2e	2	4	395.4	2.71	Yes	92.16	5	High	No

Conclusion

A new series of nabumetone derivatives were properly synthesized. Their structures were confirmed by FT-IR, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$ spectra. cytotoxic activity evaluation of the compounds against the A549 (lung) cancer cell line and WRL68 (normal) cell was performed. Among the examined compounds the most promising cytotoxic agents were (2a, 2b, and 2e) with IC_{50} values of $24.62 \mu\text{g/ml}$, $23.43 \mu\text{g/ml}$, and $24.06 \mu\text{g/ml}$ respectively compared to erlotinib. The selectivity index study from testing the compounds against normal cells showed that all the compounds are safe on normal cells and selective towards cancerous cells as shown from their high IC_{50} values ranging from ($120.2-411.9 \mu\text{g/ml}$). molecular docking for ligands interaction with EGFR protein was performed and the findings were in agreement with *In vitro* results. The ADME study results revealed that compounds (2a-2c, and 2e) have promising

pharmacokinetic properties since they are highly absorbed by GI tract and don't penetrate the BBB, they also comply with the Lipinski rule.

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References

- 1- Dawood DH, Srour AM, Saleh DO, Huff KJ, Greco F, Osborn HMI. New pyridine and chromene scaffolds as potent vasorelaxant and anticancer agents. RSC Adv. 2021;11(47):29441–52.
- 2- Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D, et al. Resistance to cancer chemotherapy: Failure in drug response from ADME to P-gp. Cancer

- Cell Int. 2015;15(1):1–13.
- 3- da Cunha Santos G, Shepherd FA, Tsao MS. EGFR Mutations and Lung Cancer. *Annu Rev Pathol Mech Dis*. 2011;6(1):49–69.
- 4- Nam Y, Hwang D, Kim N, Seo HS, Selim KB, Sim T. Identification of 1 H-pyrazolo[3,4-b]pyridine derivatives as potent ALK-L1196M inhibitors. *J Enzyme Inhib Med Chem*. 2019;34(1):1426–38.
- 5- Clemow DB, Johnson KW, Hochstetler HM, Ossipov MH, Hake AM, Blumenfeld AM. Lasmiditan mechanism of action – review of a selective 5-HT_{1F} agonist. *J Headache Pain*. 2020;21(1):71.
- 6- Tsai RS, Carrupt PA, Tayar N El, Giroud Y, Andrade P, Testa B, et al. Physicochemical and Structural Properties of Non-Steroidal Anti-inflammatory Oxycams. *Helv Chim Acta*. 1993;76(2):842–54.
- 7- Lei H, Kim JH, Son S, Chen L, Pei Z, Yang Y, et al. Immunosynodynamic Therapy Designed with Activatable Sonosensitizer and Immune Stimulant Imiquimod. *ACS Nano*. 2022;16(7):10979–93.
- 8- Najjar A, Karaman R. The prodrug approach in the era of drug design. *Expert Opin Drug Deliv*. 2019;16(1):1–5.
- 9- AbdelHaleem A, Mansour AO, AbdelKader M, Arafa RK. Selective VEGFR-2 inhibitors: Synthesis of pyridine derivatives, cytotoxicity and apoptosis induction profiling. *Bioorg Chem*. 2020;103:104222.
- 10- Abdel-Rahman AAH, Shaban AKF, Nassar IF, EL-Kady DS, Ismail NSM, Mahmoud SF, et al. Discovery of New Pyrazolopyridine, Furopyridine, and Pyridine Derivatives as CDK2 Inhibitors: Design, Synthesis, Docking Studies, and Anti-Proliferative Activity. *Molecules*. 2021;26(13):3923.
- 11- Hosseinzadeh Z, Razzaghi-ASL N, Ramazani A, Aghahosseini H, Ramazani A. Synthesis, cytotoxic assessment, and molecular docking studies of 2,6-diaryl-substituted pyridine and 3,4- dihydropyrimidine-2(1H)-one scaffolds. *TURKISH J Chem*. 2020;44(1):194–213.
- 12- R SA, Walmik P. Synthesis , antimicrobial and antioxidant activities of some indole analogues containing naphthyridine and pyrimidinonaphthyridine systems. 2012;51: 1593–606.
- 13- Achagar R, Elmakssoudi A, Thoume A, Dakir M, Elamrani A, Zouheir Y, et al. Nanostructured Na₂CaP₂O₇: A New and Efficient Catalyst for One-Pot Synthesis of 2-Amino-3-Cyanopyridine Derivatives and Evaluation of Their Antibacterial Activity. *Appl Sci*. 2022;12(11):5487.
- 14- Atla SR, Nagireddy NR, Yejella RP. Anti-inflammatory, Analgesic and Antimicrobial activity studies of novel 4,6-disubstituted-2-amino-3-cyanopyridines. 2014;1(1):51–62.
- 15- Karim HA, Raauf MR, Ali KF. Synthesis and Preliminary Pharmacological Evaluation of Some New Triazole Derivatives Bearing Nabumetone Moiety Targeting Cyclooxygenase Enzyme. 2021;12(1):190–5.
- 16- Ismail MMF, Farrag AM, Harras MF, Ibrahim MH, Mehany ABM. Apoptosis: A target for anticancer therapy with novel cyanopyridines. *Bioorg Chem*. 2020;94:103481.
- 17- Freshney RI. Culture of animal cells: a manual of basic technique and specialized applications. New Jersey: John Wiley & Sons; 2015.
- 18- Jones G, Willett P, Glen RC, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking 1 Edited by F. E. Cohen. *J Mol Biol*. 1997;267(3):727–48.
- 19- Hanyzewski G, Mull M. HERMES: a tool kit for developing distributed modelling applications. *Nanotechn-*

- ology. 1996;7(3):193–6.
- 20- Alawad KM, Mahdi MF, Raauf AMR. Molecular Docking study , and In vitro Evaluation of Antitumor Activity of Some New Isoxazoline and Pyrazoline Derivatives of Nabumetone against breast cancer cell line (MCF-7). Al Mustansiriyah J Pharm Sci. 2022;2(3):24–34.
- 21- Abourehab MAS, , Alaa M. Alqahtani BGMY and AMG. Globally Approved EGFR Inhibitors: Insights into Their Syntheses, Target Kinases, Biological Activities, Receptor Interactions, and Metabolism. Molecules. 2021;26(21):6677.
- 22- Park JH, Liu Y, Lemmon MA, Radhakrishnan R. Erlotinib binds both inactive and active conformations of the EGFR tyrosine kinase domain. Biochem J. 2012;448(3):417–23.
- 23- Raauf AMR, Omar TNA, Mahdi MF, Fadhil HR. Synthesis, molecular docking and anti-inflammatory evaluation of new trisubstituted pyrazoline derivatives bearing benzenesulfonamide moiety. Nat Prod Res. 2022;1–8.
- 24- Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:1–13.
- 25- Mazen Mohammed Jwaid, Karima Fadhil Ali, Mayada Hadi Abdalwahab. Synthesis, Antibacterial study and ADME Evaluation of Novel Isonicotinoyl Hydrazide Derivative Containing 1,3,4-Oxadiazole Moiety. Al Mustansiriyah J Pharm Sci. 2022;20(4):113–21.
- 26- Prayong P, Barusrux S, Weerapreeyakul N. Cytotoxic activity screening of some indigenous Thai plants. Fitoterapia. 2008;79(7–8):598–601.
- 27- Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2012;64