Nitrophenyl-Group-Containing Heterocycles. 2. Synthesis, Characterization, Anticancer Activity, Apoptotic Induction and Cell Cycle Arrest of Some New 5,6,7,8-Tetrahydro-isoquinolines Bearing 2-Nitrophenyl Group

Abdelreheem A. Saddik
Assiut University
Etify A. Bakhite
Assiut University
Reda Hassanien
New Valley University
Nasser Farhan
New Valley University
Eman M. Sayed
Emanmohsen@sci.nvu.edu.eg
New Valley University
Marwa Sharaky
cairo university

Research Article

Keywords: Anticancer, Apoptosis, cell cycle, Synthesis, Characterization, tetrahydrosoquinoline

Posted Date: August 20th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4493252/v2

Nitrophenyl-Group-Containing Heterocycles. 2. Synthesis, Characterization, Anticancer Activity, Apoptotic Induction and Cell Cycle Arrest of Some New 5,6,7,8-Tetrahydro-isoquinolines Bearing 2-Nitrophenyl Group

Abdelreheem A. Saddik[1], Etify A. Bakhite[1*], Reda Hassanien[2], Naseer Farhan[2], Eman M. Sayed[2, 3*], Marwa Sharaky [4]

[1]Chemistry Department, Faculty of Science, Assuit University, Assiut 71516, Egypt.

[2]Chemistry Department, Faculty of Science, New Valley University, 72511 El-Kharja, Egypt.[3] South Egypt cancer institute- Assuit university- Egypt. [4] Pharmacology Unit - Cancer Biology Department - National Cancer Institute - Cairo University.

ABSTRACT: In this work, we synthesized a new 5,6,7,8-tetrahydroisoquinolines and 6,7,8,9-tetrahydrothieno[2,3-*c*] isoquinolines based on 4-(2-nitrophenyl moiety) as anticancer drugs. These newly synthesized compounds were characterized by spectral date (FT-IR, ^{1}HNMR, ^{13}C NMR) and elemental analysis. Also, the synthesized compounds were evaluated for their anticancer activity against eight cell lines as one spot concentration, and one normal human skin fibroblasts cell line **HSF**, then use different concentrations to calculate the IC_{50} of our compounds against two selective cell lines. We found that compound **3** is the most active compound against **HEPG2** cell line. Also the most active compound against **HCT116** cell line was compound **9c.** Generally, the results revealed moderate anticancer activity for most of the tested compounds. After that we examined the impact of compound **3** on the growth of **HEPG2** cell lines, employing flow cytometry and an Annexin V-FITC apoptotic assay. Compound **3** caused cell cycle arrest at the **G2/M** with a 50-fold increase in apoptosis of **HEPG2** cell line. Finally, The molecular docking study for the two compounds **3, 7** were studied against RET enzyme. We found that compound **3** inhibit RET enzyme by **ΔG -5.2 (kcal/mol) and** compound **7** inhibit RET enzyme by **ΔG -5.6 (kcal/mol).** Compared with Standard **(alectinib)** which can inhibit RET enzyme by **ΔG -7.2 (kcal/mol).** This mean that these compounds can used as anticancer drug and RET inhibitors.

Keywords: Anticancer, Apoptosis, cell cycle, RET inhibitors Synthesis, Characterization, 5,6,7,8-Tetrahydroisoquinolines; 6,7,8,9-Tetrahydrothieno[2,3-*c*] isoquinolines.

1. INTRODUCTION

Today Cancer diseases are the leading cause of death in the world [1]. There are various types of cancer disease that require treatment, including breast cancer, lung cancer, liver cancer, colon cancer, and brain cancer. Colon cancer (CC) also named as Colorectal cancer (CRC) that is a major cause of cancer mortality and morbidity globally [2]. Especially in developed and developing countries [3]. In 2023, approximately 153,020 individuals will be diagnosed with CRC and 52,550 will die from the disease, including 19,550 cases and 3750 deaths in individuals younger than 50 years [4].

Hepatocellular carcinoma (HCC) also one of the leading cause of cancer-related deaths in the world [5]. Which usually caused by non-alcoholic fatty liver disease (NAFLD) [6]. Also, viruses' C and B can cause liver cancer [7].

Heterocyclic compounds include isoquinolines, tetrahydroisoquinolines are reported to treat liver and colon cancer diseases. For example, for treatment colon cancer by Thiophene Heterocyclic Compound [3], 6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid [8], benzimidazole-isoquinolinones [9], Tetrahydroisoquinoline Derivatives [10]. Also liver cancer can treatment by 3-arylisoquinoline [11], 1-styrenyl isoquinoline [12].

Isoquinolines, tetrahydroisoquinolines compounds were reported to has various biological activities. including antimicrobial [13], anti-oxidant [14], anti-inflammatory [14,15], antipyretic [15], antihypertensive [16], antitumor [17-25].

5,6,7,8-Tetrahydroisoquinoline ring is a structural moiety of many alkaloids [26, 27]. That include potent cytotoxic agents display a range of antitumor activities [23, 24]. On the other hand, many nitro-group-containing compounds are reported to possess many applications in the fields of biochemistry and medicine [28-31]. In view of the above observations and as a continuation of our previous work on tetrahyroisoquinolines [32,

33]. The current work was planned to synthesize and characterize a new 5,6,7,8-tetrahyroisoquinolines compounds with the hope that these new compounds will find good applications in both biological and medicinal fields owing to their incorporation of various pharmacophores. Also, the study these synthesized compounds as anticancer against two cell line **HEGP2** (Liver cell line) and **HTC116** (Colon cell lines) have been carried out. And the obtained results are reported herein. Moreover, we study the mechanism of cell cycle arrest and apoptosis percent when treat **HEGP2** cells with compound **3**. Finally, we apply the molecular docking study to some compounds to find the enzyme that can react with our compounds.

2. RESULTS AND DISCUSSION

2.1. Synthesis

The starting materials, 7-acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(2-nitrophenyl)-5,6,7,8-tetrahydrosoquinoline-3(2*H*)-thiones **2** were prepared *via* cyclocondensation of 2,4-diacetyl-5-hydroxy-5-methyl-3-(2-nitrophenyl) cyclohexanones **1** with 2-cyanothio-acetamide by refluxing in ethanol containing pipridine as a basic catalyst in analogy to the reported procedures (Scheme 1) [26-29].

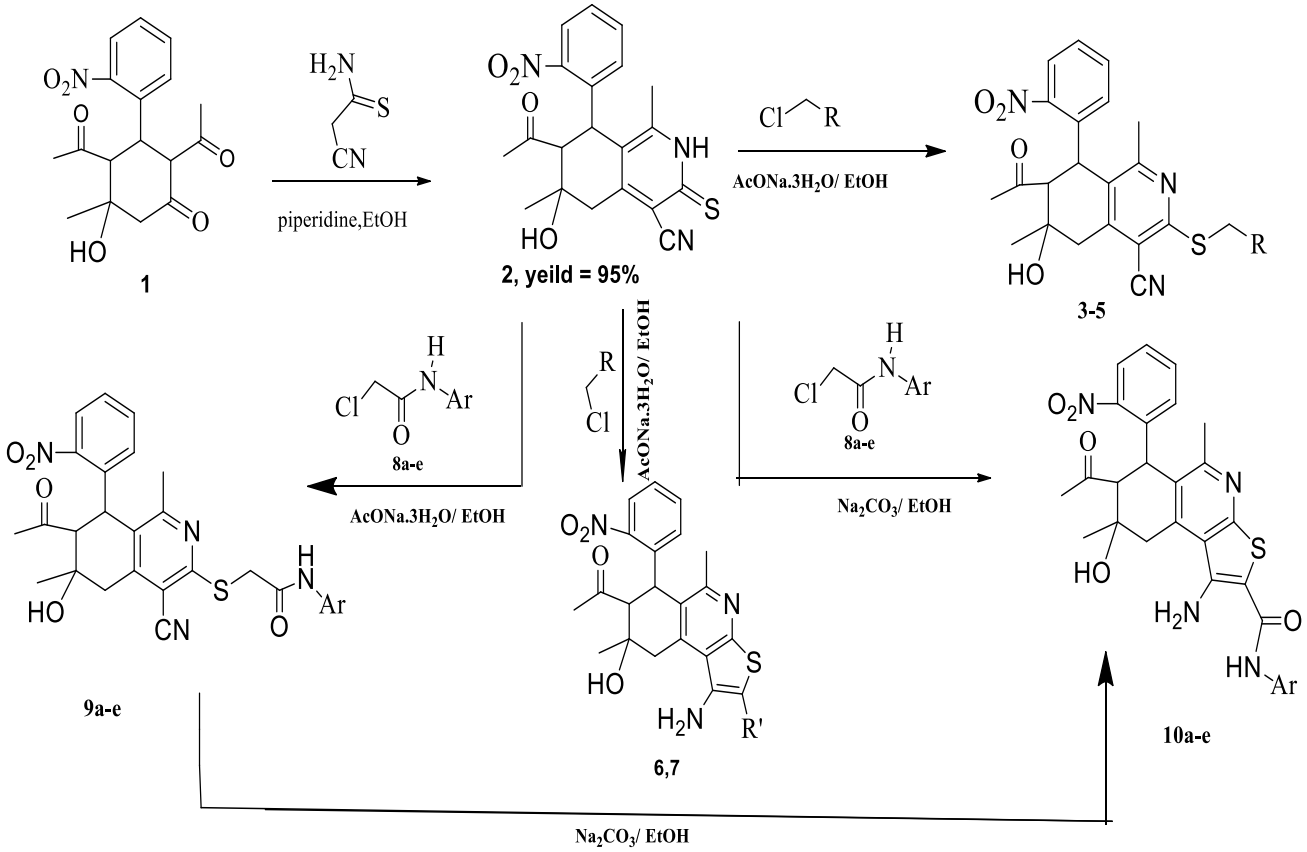
Treatment of compound **2** with some halocompounds namely; ethyl iodide, ethyl chloroacetate or 2-chloroacetamide by refluxing in ethanol, in the presence of slightly excess molar amounts of sodium acetate trihydrate, for one hour led to the formation of *S*-substituted thio-5,6,7,8-tetrahydroisoquinoline-4-carbonitriles **3**, **4,** and **5** respectively (Scheme 1). Cyclization of compounds **4** into the corresponding 2-funcutionally substituted 1-amino-6,7,8,9-tetrahydroisoquinolines **6** achieved by heating with catalytic amounts of sodium ethoxide in abs. ethanol for 5 min. (Scheme 1).

In a similar manner, reaction of compound **2** with *N*-aryl-2-chloroacetamides **8a-e** under the same (above) conditions afforded the corresponding *N*-aryl-(5,6,7,8-tetrahydroisoquinolin-3-ylthio) acetamides **9a-e** in excellent yields. Cyclization of compounds **9a-e** into the corresponding 7-acetyl-1-Amino-*N*-aryl-5,8-dimethyl-8-hydroxy-6-(2-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3-*c*] isoquinoline-2-carboxamides

10a-e was acheived by heating with catalytic amounts of sodium ethoxide in abs. ethanol for 5 mins. Compounds **10a-e** were also synthesized *via* heating compound **2** with the respective *N*-aryl-2-chloroacetamides **8a-e** in abs. ethanol in the presence of slightly excess molar amounts of sodium carbonate for 60 min. (Scheme **1**). Conversion of **9a-e** into the corresponding **10a, d** obeys intramolecular Thorpe-Ziegler cyclization which its mechanism is outlined before in our publication [30].

In contrast, reaction of **2** with chloroacetonitrile under the same (above) conditions yielded 1-amino-*2*-cyano-6,7,8,9-tetrahydrothieno[2,3-*c*] isoquinoline **7** directly (Scheme **1**). For full result see supporting data S1-S35.

Scheme 1. Synthesis of compounds 2-7, **9a-e** and **10a,d**



Compd.	R	Yeild %
3	CH_3	87%
4	CO_2Et	91%
5	$CONH_2$	95%

Compd.	R'	Yeild%
6	$COOCH_2CH_3$	89%
7	CN	93%

Compd.	8,9 Ar	Yeild%
a	C6H5	96%
b	C_6H_4Me (2)	94%
c	C_6H_4COMe (2)	96%
d	C_6H_4Cl (2)	92%
e	C_6H_4OMe (2)	89%

Compd.	10 Ar	Yeild%
a	C6H5	93%
d	C_6H_4Cl (2)	94%

2.2. Cytotoxic activity

2.2.1. Cytotoxic activity

The inhibition activity of all compounds **3-7, 9a-e and 10d** was tested as one concentration spot 100 µg/ml against eight human cancer cell lines (Figure1) (human liver carcinoma **HEPG2**, **and HUH7**, human breast carcinoma **MCF7**, human colon carcinoma **HCT116** and **CACO2**, human lung carcinoma **H460** and **A459,** and human

osteosarcoma **MG-63** and on normal human skin cell line **HSF**). All cell lines were obtained from national cancer institute, Cairo - Egypt:

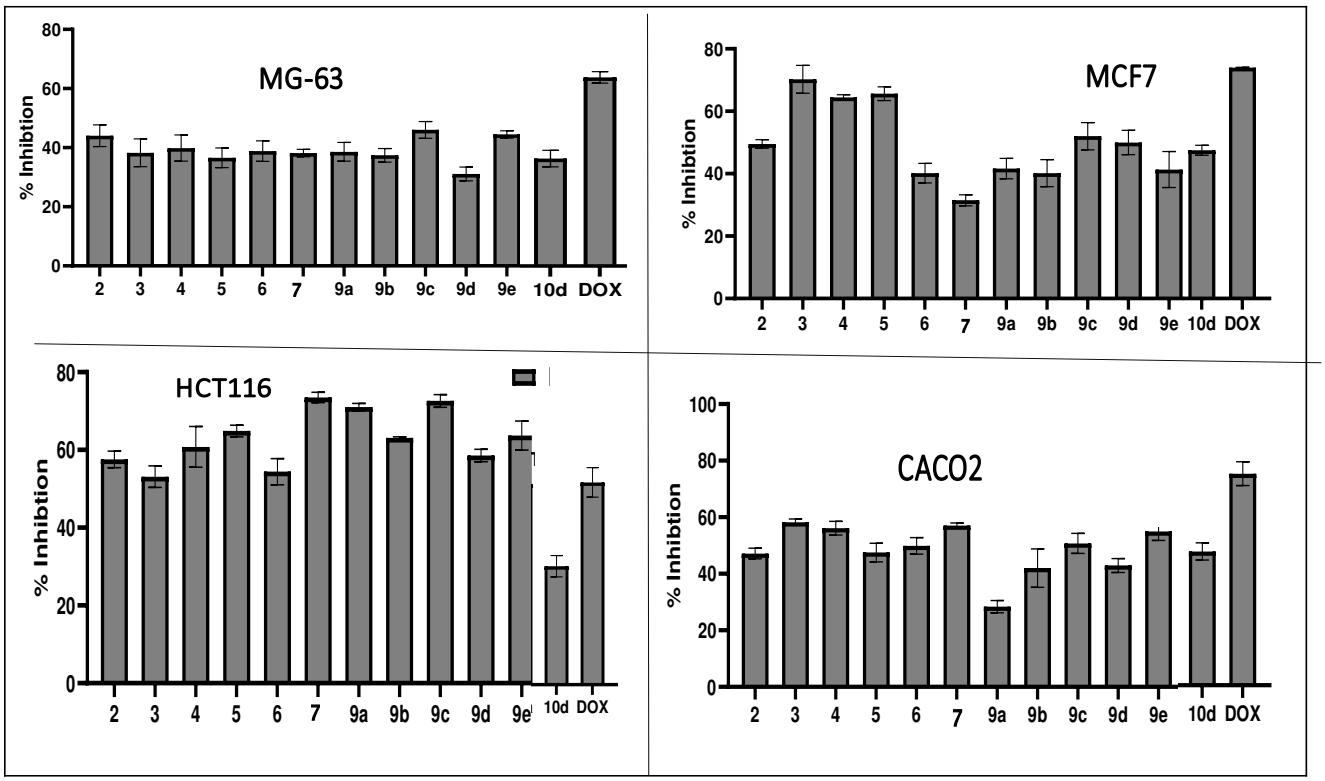
From the activity of theses compound aganist normal human skin cell line **HSF** show that compound **4, 5 , 9c, 9d** and **9e** have inhabition percent aganist normall cell line less than Doxorubicin it self this indicat that these compounds more safe and specific than douxrbin it self. (Table 1) for more test details and for the raw data see supporting information Table S1.

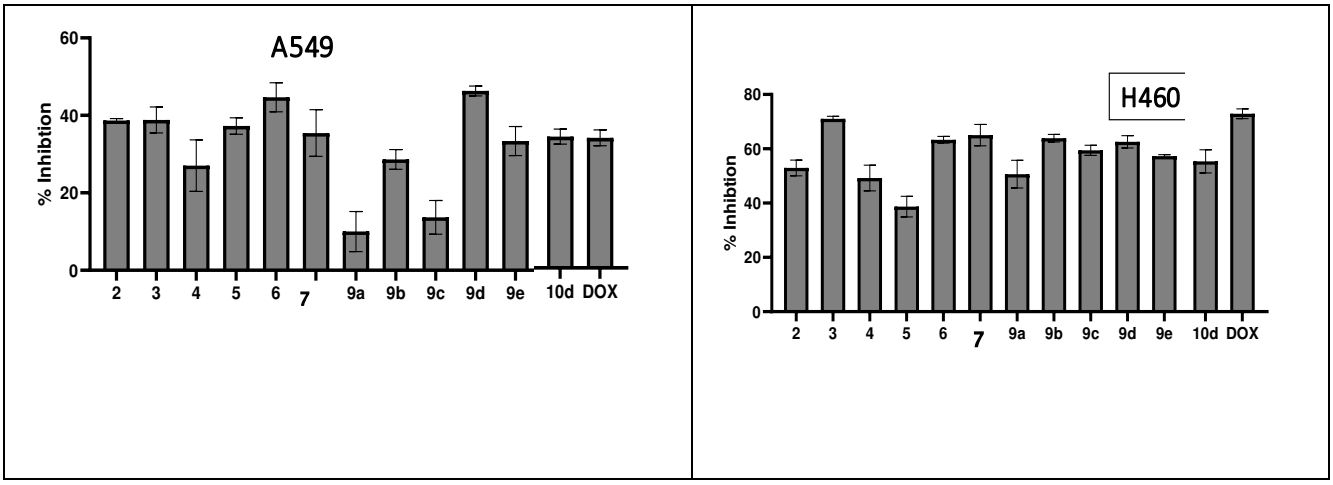
Then two cell line **HEPG2** and **HCT116** has evaluated *in vitro* at different concentrations ranged from 0 to 100 µg/ml using the MTT assay method. In this work, doxorubicin was used as a positive control drug for comparison purposes with the drug candidates **3-7, 9a-e and 10d** under the same experimental conditions. Different concentrations of these compounds were tested to reach the concentration which could cause death for 50 % of the cancer cells after 48 hr.

The results obtained revealed that among all tested compounds: (i) four compounds **3, 6, 4** and **10** showed the most active cytotoxic activity against **HEPG2** with IC_{50} of 31,40,42 and 42 µg/ml respectively (Fig. 2) (Table 2) compared with (Doxorubicin standard drug). for the raw data see supporting information Table S2,4 (FigureS34).

, (ii) and three compounds **7**, **9a and 9c** showed considerable cytotoxic activity against **HCT116** with IC_{50} 50, 55 and 49 µg/ml respectively (Fig. 3). also (Figure 4, Table 2) show IC_{50} of all synthesized compounds compared with (Doxorubicin standard drug). for the raw data see supporting information Table S3,5 (FigureS35).

.


MG-63
% Inhibtion
0
20
40
60
80
2 3 4 5 6 7 9a 9b 9c 9d 9e 10d DOX
MCF7
% Inhibtion
0
20
40
60
80
2 3 4 5 6 7 9a 9b 9c 9d 9e 10d DOX
HCT116
% Inhibtion
0
20
40
60
80
2 3 4 5 6 7 9a 9b 9c 9d 9e 10d DOX
CACO2
% Inhibtion
0
20
40
60
80
100
2 3 4 5 6 7 9a 9b 9c 9d 9e 10d DOX


A549
% Inhibtion
0
20
40
60
2 3 4 5 6 7 9a 9b 9c 9d 9e 10d DOX
H460
% Inhibtion
0
20
40
60
80
2 3 4 5 6 7 9a 9b 9c 9d 9e 10d DOX

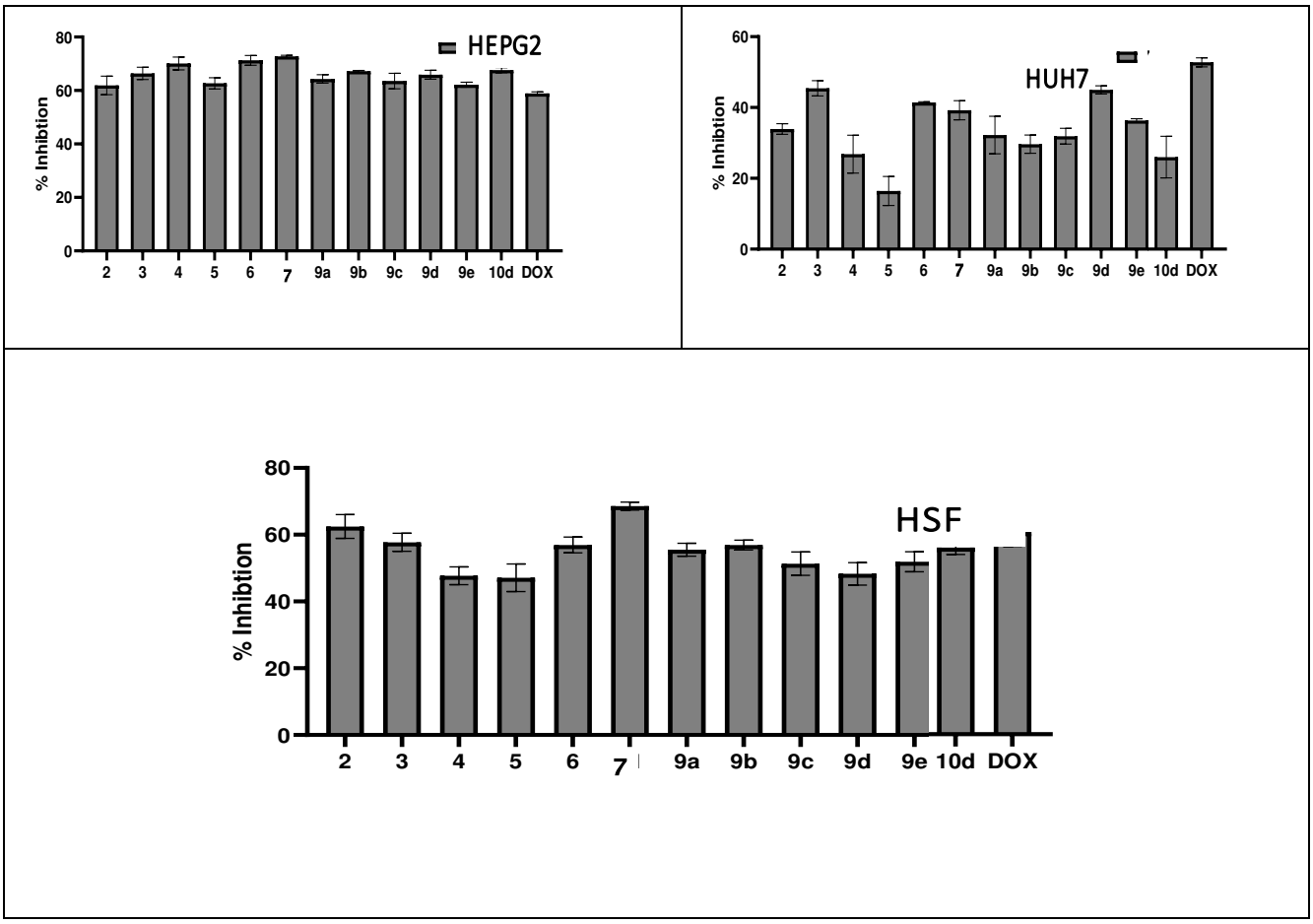


Figure1: Inhibition percent of one spot 100 µg/ml concentration of each synthesized compounds against eight cell lines in compared with Doxorubicin.

Table 1: Inhibition activity in one spot 100 µg/ml concentration of all compounds against normal skin cell line **HSF** in compared with Doxorubicin.

Compd.no.	Inhibition percent of **HSF** cell line	Compd.no.	Inhibition percent of **HSF** cell line
2	65	**9b**	58
3	60	**9c**	48
4	50	**9d**	50
5	50	**9e**	53
6	57	**10d**	57

7	70	**Doxorubicin**	57
9a	57		

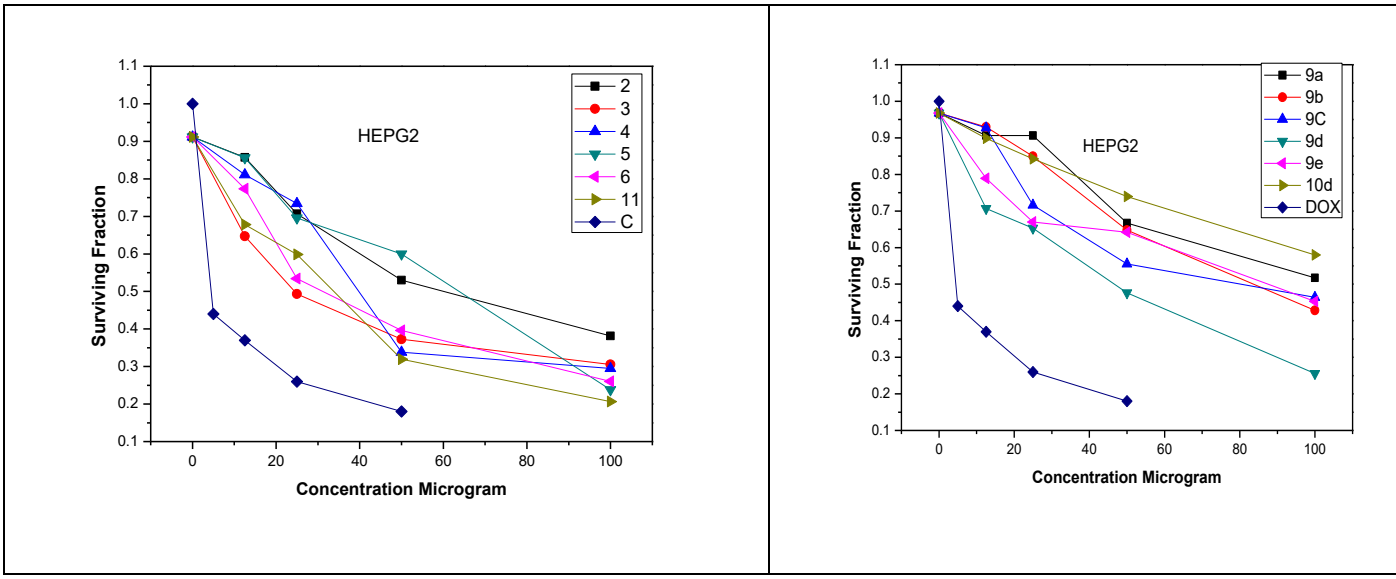


Figure 2: Cell viability of compounds **2-6,9a-e,9a** and **9d** against **HEPG2** cell lines.

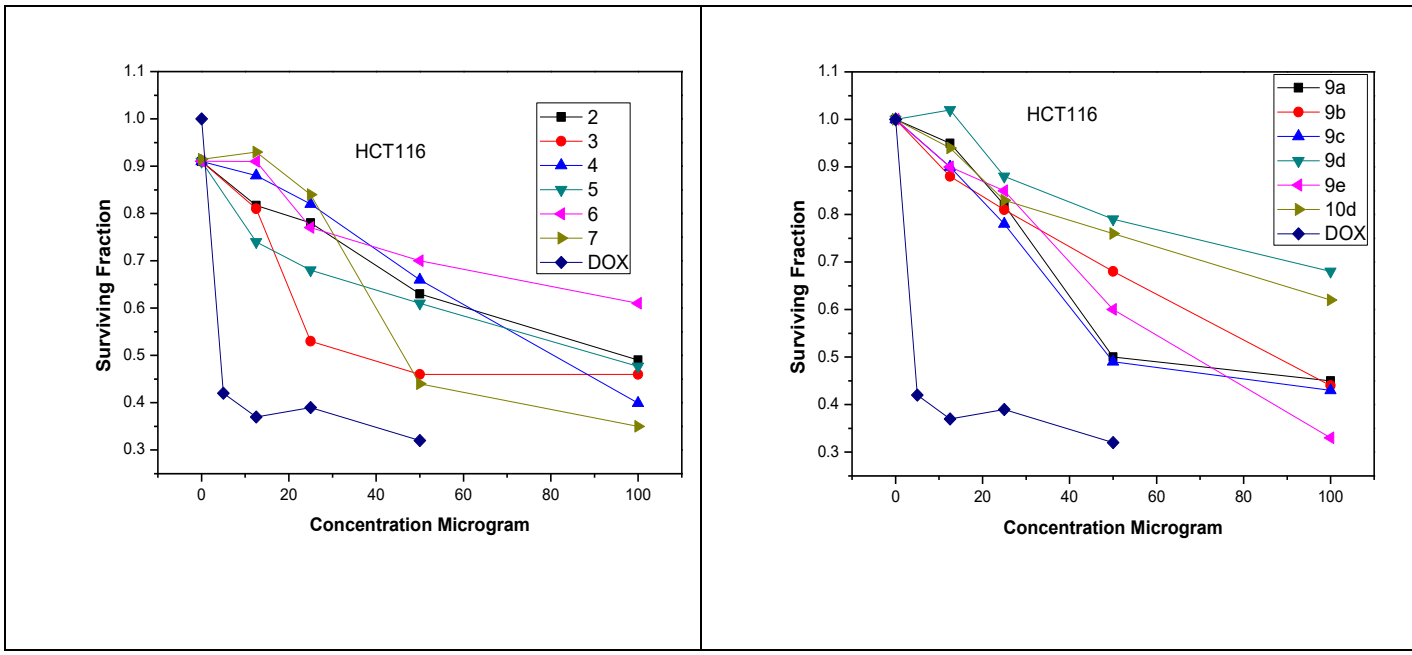
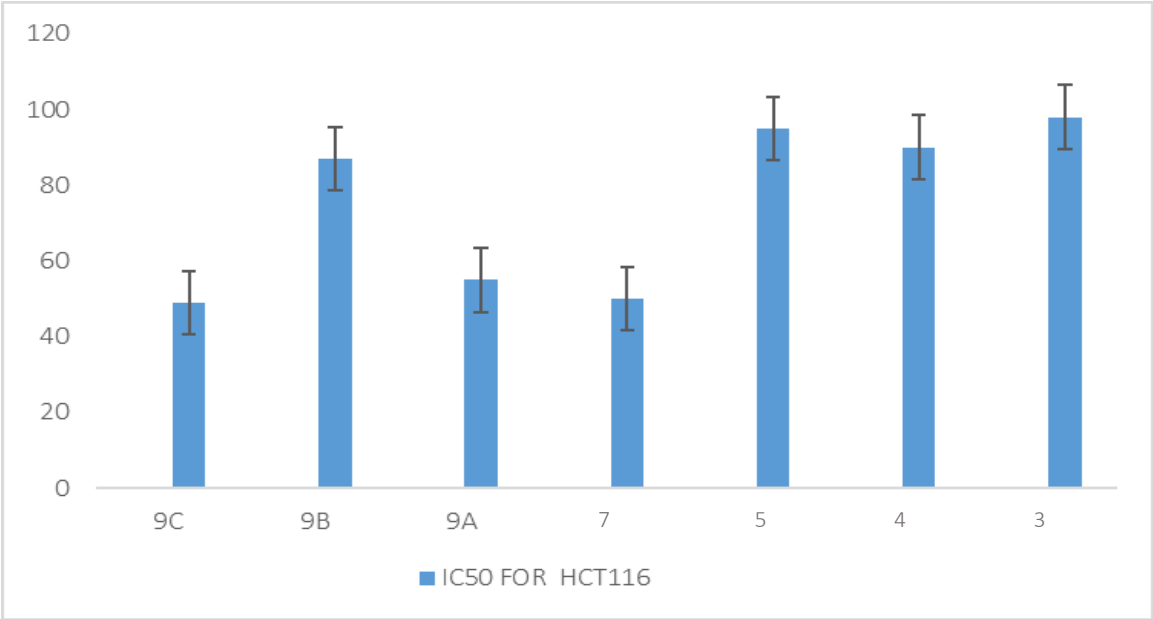


Figure 3: Surviving fraction of compounds **2-6, 9a-e** and **10d** against **HCT116** cell lines.

Table 2: IC_{50} of all synthesized compounds against two cell line **HCT116** and **HEPG2**.

Compd.no.	IC_{50}±S.D. μg/ml against **HCT116**	IC_{50}±S.D. μg/ml against **HEPG2**
2	>100	47±0.028
3	98± 0.083	31±0.013
4	90±0.022	42±0.024
5	95±0.036	76±0.095
6	>100	40±0.029
7	50±0.031	38±0.026
9a	55±0.014	>100
9b	87±0.048	87±0.058
9c	49±0.081	85±0.032
9d	>100	48±0.055
9e	67	92
10d	>100	>100
DOX	4.19	4.58



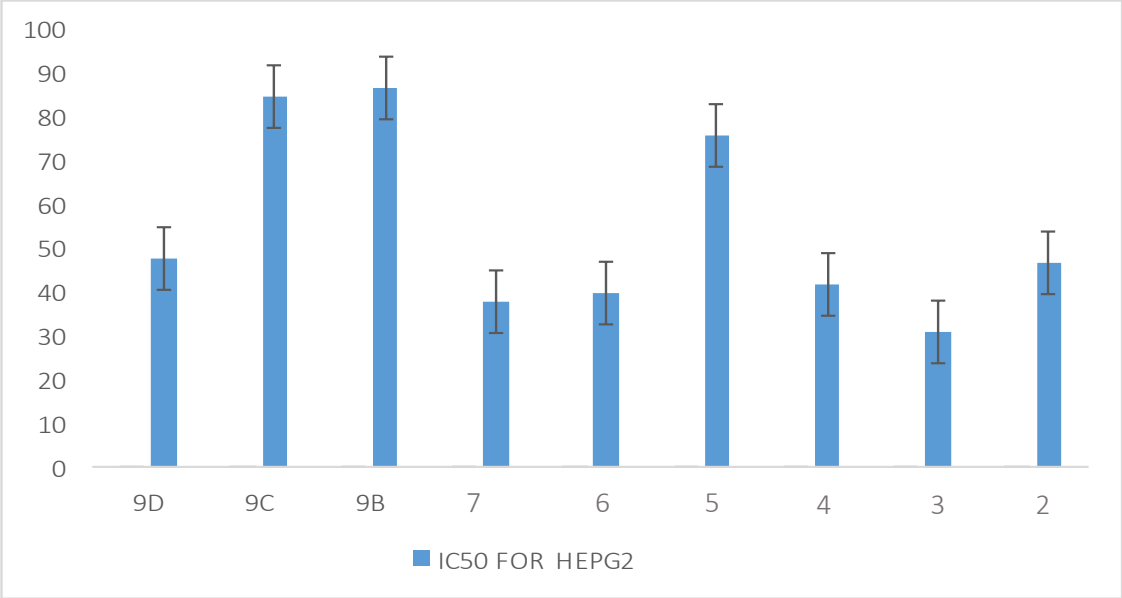


Figure 4: **IC_{50}** of all synthesized compounds against two cell line HCT116 and HEPG2 with the S.D. error bar.

2.2.2. Cell cycle arrest of HEPG2 Cells

To examine the growth inhibition mechanism of compounds **3** in relation to cell cycle progression and regulation in **HEPG2** cancer cells, we investigate the growth inhibitory cell cycle mechanism of **HEPG2** cell lines after adding compound **3** using flow cytometry. The impact on cell cycle distribution was assessed by a DNA flow cytometry analysis, through the incubation of a **HEPG2** cell with compound **3** at its IC_{50} concentration (31 µg/ml) for 48 h (Figure 2). From the obtained results, it was found that: (i) **HEPG2** cells exposed to compound **3** arrested at the G2/M phase of the cell cycle, with the G2/M phase fraction increasing from 12.12% (in control cells) to 19.45% after being treated with compound **3** (Figure 5 and Table 3).

Table 3. Cell cycle analysis of **HEPG2** treated with compound **3**.

code	%G0-G1	%S	%G2/M
3/HEPG2	**32.45**	**23.4**	**19.45**
Cont.HEPG2	**34.54**	**23.51**	**12.12**

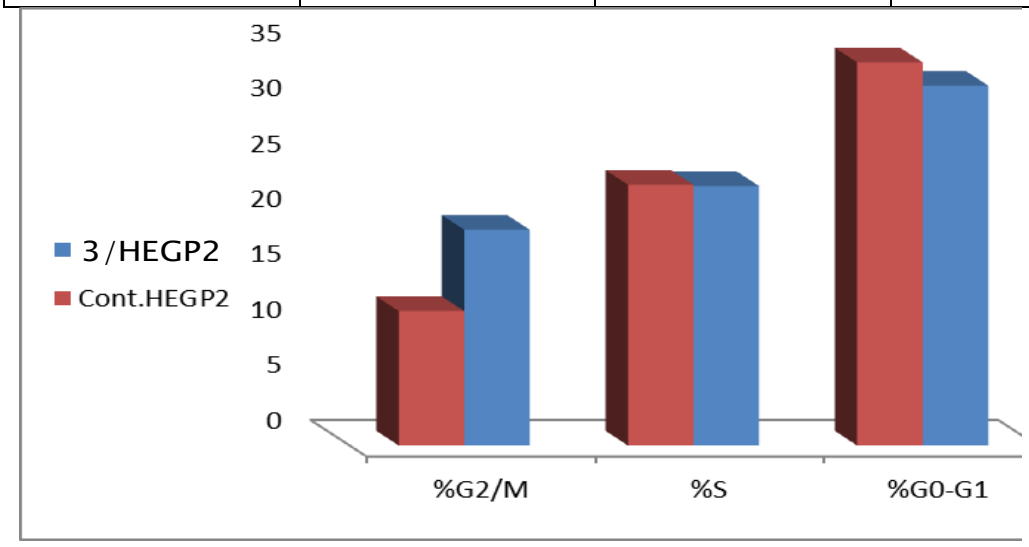


Figure 5: Cell cycle analysis of **HEPG2** cells treated with compounds **3**.

2.2.3. Induced apoptosis

Apoptosis induction is another approach to study the anti-proliferative action of compound **3** against **HEPG2** cells, which demonstrates an increased cell population in G2/M phases in compound 3-treated **HEPG2** cells.

The Annexin V-FITC/PI assay results indicated that: (i) compound **3** treatment of **HEPG2** cells resulted in early and late cellular apoptosis, as evidenced by a significant increase in the percentage of apoptotic cells in both the early apoptotic phase (from 0.31% to 15.3%) and the late apoptotic phase (from 0.20% to 11.41%), indicating a high increase in total apoptosis when compared to the untreated control. Also, the number of necrosis cells increased from 1.36% to 3.10% (Figures 6 and 7). The aforementioned results show a 50-fold increase in in **HEPG2** cellular apoptosis after treatment with compounds **3**. So compound **3** possesses a biological mechanism that inhibits **HEPG2** cell development, resulting in an anticancer cytotoxic impact.

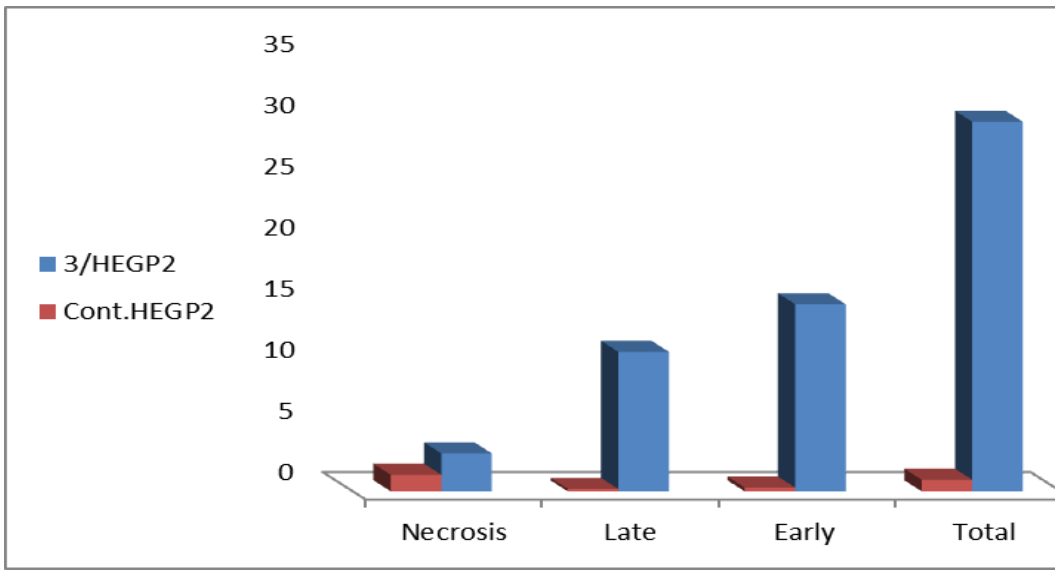


Figure 6: Apoptosis/necrosis assessment of **HEPG2** cells after treatment with compounds **3**. Different cell populations were plotted as a percentage of total events. Data are presented as mean ± SD; n =3.

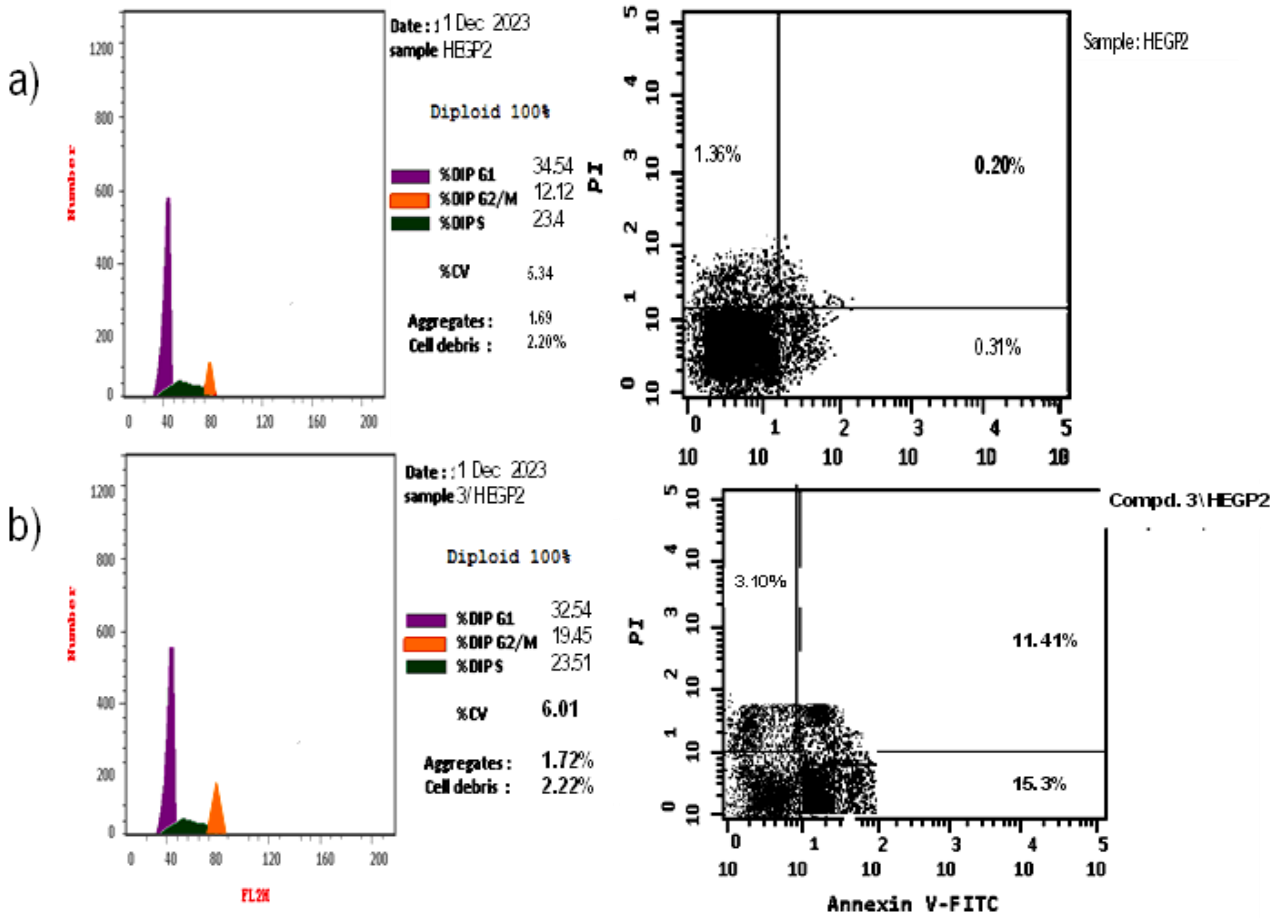


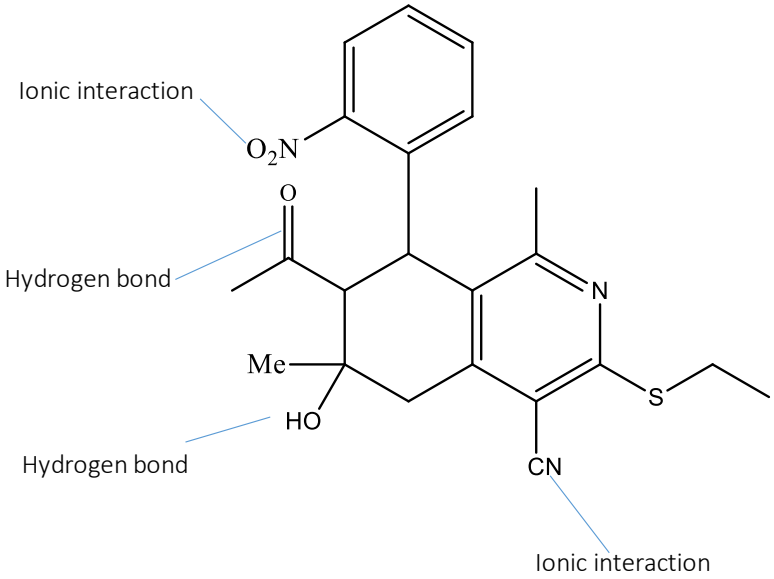
Figure 7: Apoptosis of **HEPG2** after treatment with compounds **3.** (a) Control HEPG2. (b) Compound **3/HEPG2.**

2.2.4. Structure action relationship(SAR)

5,6,7,8-tetrahydroisoquinoline is consist of two ring pyridine ring and unsaturated benzene ring which enrich the antiprofilate activity also the substitution in position C8 with OH group and C7 with AC group can make hydrogen bond with the enzymes responsible for the cancer disease such as DHFR, CDK2, Eef2, tubulin, EGFR…. Also the NH_2 substitution in C1 position in the cyclized compounds give hydrophobic interaction with the enzymes. Moreover, the nitro phenyl substitution the nitro group is strong with drawing group which can make ionic bond with a donor group in the enzymes. Theses reason prove that our compounds can inhibit the enzymes responsible for cancer disease by several bonds. And from the previous study we show that tetrahdroisoquinoline is potent anticancer drugs against [24, 32, 33] breast cancerous cell,

lung cancerous cell line, pancreatic cancerous cell line, liver cancerous cell line, colon cancerous cell line. Moreover, isoquinoline is commonly used as anticancer drug such as papaverine, quinapril.

Scheme 2: SAR study of interaction position of isoquinoline compound **3**.



2.2.5. **Molecular docking against RET enzyme.**

Molecular docking studies were performed in **(I Mole Lab for Bioinformatics-Cairo).**

The molecular docking results presented in Table 4 provide valuable insights into the binding affinities of various ligands with the RET (Rearranged during Transfection) tyrosine kinase receptor. It is important to note that RET is a tyrosine kinase receptor, playing a crucial role in cell signaling pathways involved in cell growth, differentiation, and survival. The biological activity of these compounds as potential RET inhibitors is of particular interest due to the involvement of RET in various cancers, particularly thyroid cancer and some forms of lung cancer.

The Gibbs free energy (ΔG) values obtained from the docking simulations offer a quantitative measure of the ligand-protein interactions, with more negative values indicating stronger binding.

Compounds **3** and **7**, have important binding energies interactions with the **RET** receptor. Compound **3** forms a conventional hydrogen bond with ILE890 and several hydrophobic interactions, while compound **7** interacts through a hydrogen bond with ARG789 and a Pi-Cation interaction with ARG854

Also the standard compound (alectinib) exhibits interactions, primarily relying on a carbon hydrogen bond and hydrophobic interactions.

Table 4: ΔG (kcal/mol) for each ligand with protein (RET).

Ligand	**ΔG (kcal/mol)**
3	**-5.2**
7	**-5.6**
Standard (alectinib)	**-7.2**

Table 2: 3D and 2D for (3,7 and Standard) ligands against RET enzyme.

Compound 3 interaction

Interaction	Distance	Category	Type
A:ILE890:HN - :UNL1:N	2.45117	Hydrogen Bond	Conventional Hydrogen Bond
A:VAL892:CG2 - :UNL1	3.78473	Hydrophobic	Pi-Sigma
A:VAL892 - :UNL1	4.54808	Hydrophobic	Alkyl
A:PRO934 - :UNL1	4.45101	Hydrophobic	Alkyl
:UNL1 - A:VAL892	5.00294	Hydrophobic	Pi-Alkyl
:UNL1 - A:LEU900	5.10055	Hydrophobic	Pi-Alkyl

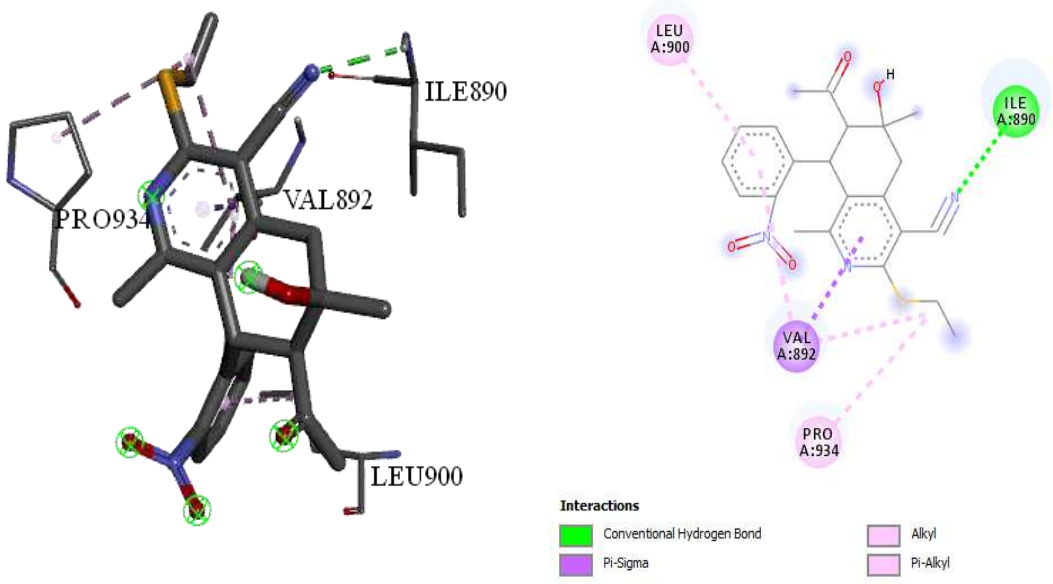
Compound 7 interaction

Interaction	Distance	category	Type
A:ARG789:HH21 -	2.93466	Hydrogen Bond	Conventional Hydrogen

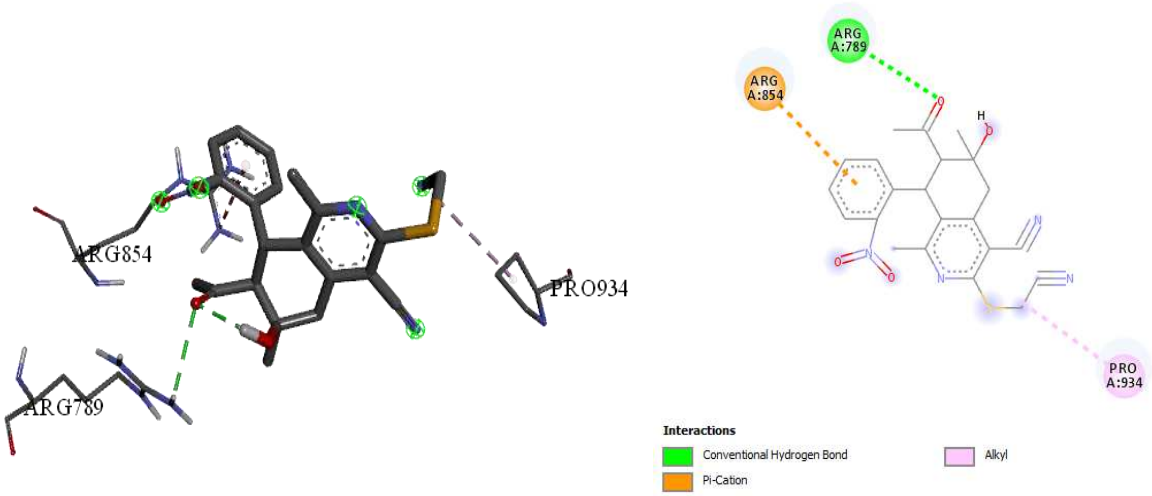
:UNL1:O			Bond
A:ARG854:NH1 - :UNL1	4.33613	Electrostatic	Pi-Cation
A:PRO934 - :UNL1	4.40121	Hydrophobic	Alkyl

Standard interactions

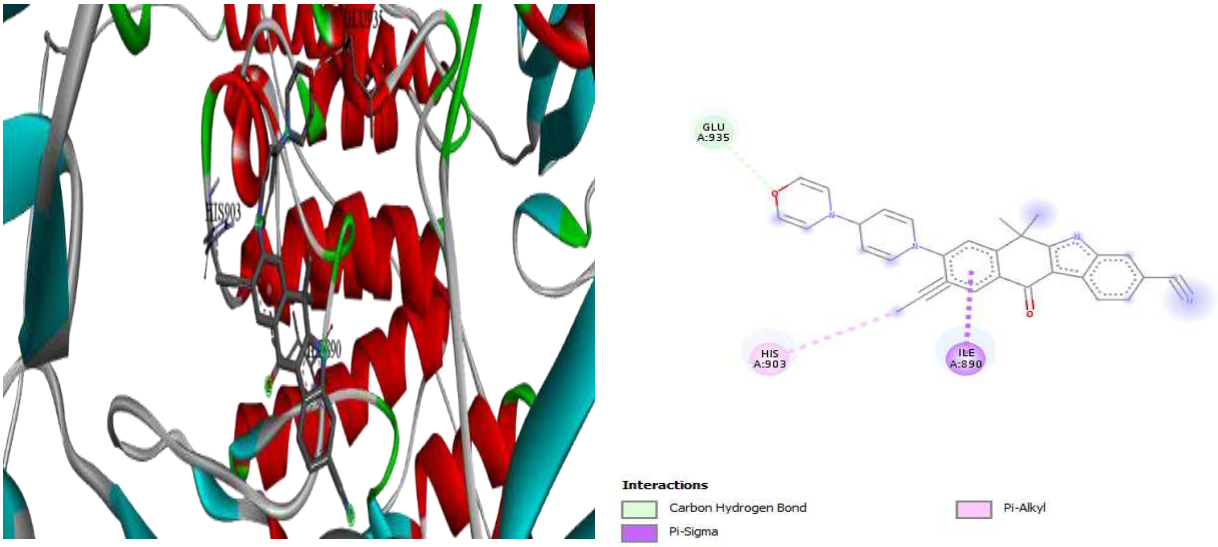
Interaction	Distance	category	Type
A:GLU935:CA - :UNL1:O	3.21679	Hydrogen Bond	Carbon Hydrogen Bond
A:ILE890:CD1 - :UNL1	3.38069	Hydrophobic	Pi-Sigma
A:HIS903 - :UNL1:C	4.53641	Hydrophobic	Pi-Alkyl



3D, 2D interactions of Compound **3** with RET receptor.



3D, 2D interactions of Compound **7** with RET receptor.



3D, 2D interactions of standard compound (alectinib) with RET receptor.

3. EXPERIMENTAL

3.1. Instrumentations

Melting points were measured using a Gallan-Kamp device and are uncorrected. The compounds' purity was confirmed by TLC and spectroscopic examination.

IR spectra were obtained using a Shimadzu 470 IR-spectrophotometer (KBr; vmax in cm-1). The ^{1}HNMR and 13C NMR spectra were obtained on a Varian A5 500 MHz spectrometer with DMSO-d6 as the solvent and tetramethylsilane (TMS) as the internal reference. Coupling constants (J values) are expressed in Hertz. Elemental studies were carried out using a Perkin Elmer 2400 LS Series CHN/O analyzer.

Chemicals used in this work include ortho nitrobenzaldhyde, cyanothioacetamide, piperidine, methyl iodide, ethyl chloroacetate, 2-chloroacetamide, chloroacetonitrile, ethanol, and sodium acetate.3H2O, sodium carbonate) were acquired from Sigma Aldrich CO.

All the cancer eight cell lines (human liver carcinoma **HEGP2**, **and HUH7**, human breast carcinoma **MCF7**, human colon carcinoma **HCT116** and **CACO2**, human lung carcinoma **H460** and **A459,** and human osteosarcoma **MG-63** and on normal human skin cell line **HSF**) were obtained from national cancer institute, Cairo - Egypt:

3.2.1. 7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(2-nitrophenyl)-5,6,7,8-tetra-hydroisoquinoline-3(2*H*)-thione (2).

A mixture of compound **1** (3. 3 g, 10 mmol), 2-cyanothioacetamide (1.0 g, 10 mmol) and piperidine or morpholine (2 mmol) in ethanol (100 mL) was refluxed for 2 h. The yellow crystals that formed on hot were collected, washed with methanol, dried in air to give compound **2**. Yield: 95 %; m. p: 279-280 °C. IR: 3411 (O-H); 3278 (N-H); 2979, 2922 (C-H, sp^2); 2219 (C≡N); 1690 (C=O, acetyl). ^{1}H NMR: δ 13.82 (s, 1H, NH); 7.3-7.82 (dd, *J* = 32, 29 Hz, 4H, 4Ar-H); 4.95- 5.03(d, *J* =28 Hz, 2H, OH, C^{8}H), 3.08-3.11 (d, *J*

=16.0 Hz, 2H, C^5H, C^7H), 2.82-2.85 (d, *J* =17.5 Hz, 1H, C^5H); 1.94 (s, 6H, CH_3, $COCH_3$);1.25 (s, 3H, CH_3). Anal. calcd for **$C_{20}H_{19}N_3O_4S$** (397. 1): C, 60.44; H, 4.82; N, 10.57, Found C, 60.70; H, 4.80; N, 10.78**%.**

Reaction of compound 2 with ethyl iodide, ethyl chloroacetate, 2-chloroacetamide or its *N*-aryl-2-chloroacetamides 8a-e; Synthesis of compounds 3, 4, 5 and 9a-e; general method.

A mixture of **2** (10 mmol), appropirate halocompound (10 mmol) and sodium acetate trihydrate (1.50 g, 11 mmol) in ethanol (100 mL) was refluxed for one hour. The solid that formed after cooling was collected and then recrystallized from ethanol to give white crystals of compounds **3, 4, 5** and **9a-e**.**5.**

3.2.2. 7-Acetyl-4-cyano-1, 6-dimethyl-3-ethylthio-6-hydroxy-8-(2-nitrophenyl)-5,6, 7,8-tetrahydroisoquinoline (3): It was synthesized by reaction of **2** with ethyl iodide. Yield: 87 %;168-170 m. p.: °C. IR: 3515 (O-H); 3066-2965 (N-H); 2932-2896 (C-H, sp^2); 2217 (C≡N); 1705 (C=O, acetyl). ^{1}H NMR: 7.19-7.82 (dd, *J* = 18 Hz, 17 Hz, 4H, 4Ar-H); 5.09 (d, *J* = 30 Hz, 1H, OH), 4.96- (s, Hz, 1H, C^8H), 3.12-3.19 (dt, *J* =12.6, 6.8 Hz, 4H, SCH_2, C^5H, C^7H), 2.82-2.87 (dd, *J* =17.2, 5.5 Hz, 1H, C^5H); 1.95-2.02 (m, 6H, $COCH_3$, CH_3);1.23 (s, 6H, 2 CH_3). Anal. calcd for **$C_{22}H_{23}N_3O_4S$** (425.1): C, 62.10; H, 5.45; N, 9.88. Found C, 61.85; H, 5.87; N, 10.10 %**.**

3.2.3. Ethyl 2-[(7-acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(2-nitrophenyl)-5,6,7,8-tetra-hydroisoquinolin-3-yl)thio]acetate (4): It was synthesized by reaction of **2** with ethyl chloroacetate. Yield: 91%; m. p.: 180-182°C. IR: 3445 (O-H); 2992(N-H); 2968, 2944 (C-H, sp^2); 2214 (C≡N); 1728,1714 (C=O, acetyl). ^{1}HNMR : 7.62 (s, *J* = 8.0 Hz,1H, Ar-H); 7.60 (d, *J* = 7.6 Hz , 1H, Ar-H); 7.52(d, *J* = 8.0 Hz , 1H, Ar-H); 7.19-(d, *J* = 7.8 Hz, 1H, Ar-H); 5.13 (d, *J* = 9.7 Hz, 1H, OH) , 4.95(d, *J* = 33.2 Hz, 1H, C^8H),4.05-4.09(m, 4H, SCH_2, OCH_2) 3.16(t, *J* =12.6 Hz , 2H, C^5H, C^7H), 2.90 (d, *J* = 17.3 Hz, 1H, C^5H);1.95 (sd, *J* = 18, 16 Hz 6H,CH_3, $COCH_3$), 1.30 (m, 3H, CH3), 1.13(t, *J* = 7.1 Hz, 3H, CH3).

^{13}C NMR (101 MHz, DMSO) δ 209.16, 168.94, 151.09, 149.34, 137.30, 134.13, 131.62, 129.01128.8, 125.31, 115.31, 104.52, 68.13, 65.05, 61.42, 43.48, 32.53, 31.84, 28.07, 24.03, 14.44.

Anal. calcd for **$C_{24}H_{27}N_3O_6S$** (485.2): C, 59.37; H, 5.60; N, 8.65. Found C, 57.95; H, 5.77; N, 8.80%**.**

3.2.4. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(2-nitrophenyl)-5,6,7,8-tetra-hydroisoquinolin-3-yl)thio]acetamide (5): It is synthesized by reaction of **2** with chloroacetamide. Yield: %;95 m.p.:210-211 °C. IR: IR: 3426 (O-H); 3312 (N-H); 2965 (C-H, sp^2); 2222 (C≡N); 1711 (C=O, acetyl). ^{1}HNMR : δ 7.82 (s, *J* = 8.0 Hz,1H, 1Ar-H); 7.57 -7.48 (d, *J* = 7.6 Hz , 4H, 2Ar-H, NH_2); ; 7.15-(d, *J* = 10 Hz , 1H, 1Ar-H); 5.09 (d, *J* = 9.7 Hz. 2H, OH, C^8H), 3.86(m, 2H, C^5H, C^7H,), 3.15(dd, *J* =12.6,15, 2H, SCH_2), 2.84 (d, *J* = 17.3 Hz, 1H, C^5H), 1.98 (s, 6H, $COCH_3$, CH3), 1.25(s, 3H, CH3).

Anal. calcd for $C_{22}H_{22}N_4O_5S$ (454.13): C, 58.14; H, 4.88; N, 12.33%. Found: C, 58.55; H, 4.95; N, 12.49%.

Reaction of compound 2 with ethyl chloroacetate, 2-chloroacetonitrile; Synthesis of compounds 6, 7.

A mixture of **2** (10 mmol), appropirate halocompound (10 mmol) and sodium carbonate (10 mmol) in ethanol (30 mL) was refluxed for one hour. The solid that formed after cooling was collected and then recrystallized from ethanol to give white crystals of compounds **6,7.**

3.2.5. **Ethyl 7-acetyl-1-amino-8-hydroxy-5,8-dimethyl-6-(2-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3-c]isoquinoline-2-carboxylate (6):** It was synthesized by reaction of **2** with ethyl chloroacetate. Yield: 88%; m.p.: 285-286°C. IR: 3425 (O-H); 3343(N-H); 2981(C-H, sp^2); 1728,1715,1705 (C=O, acetyl). ^{1}H NMR : 10.45(s, IH, NH), 7.83 (s, *J* = 10.0 Hz, 1H, Ar-H), 7.69 (d, *J* = 7.6 Hz, 2H, 2Ar-H); 7.60(d, *J* = 8.0 Hz , 1H, Ar-H); 7.19-(d, *J* = 7.8 Hz , 2H, NH_2); 5.13 (d, *J* = 9.7 Hz 1H, OH) , 4.95(d, *J* = 33.2 Hz, 1H, C^6H),4.05-4.09(m, 4H, C^9H, C^7H , CH_2 acetate) 3.16(d, *J* =12.6, 6.8 Hz , 1H, C^9H),

2.90 (d, *J* = 17.3 Hz, 3H, CH_3);1.95 (sd, *J* = 18.5 Hz, 3H, CH_3, $COCH_3$), 1.30 (m, 3H, CH3), 1.13(t, *J* = 7.1 Hz, 3H, CH3).

Anal. calcd for **$C_{24}H_{27}N_3O_6S$** (485.2): C, 59.37; H, 5.60; N, 8.65. Found C, 58.75; H, 5.69; N, 8.92 %**.**

3.2.6. 7-acetyl-1-amino-8-hydroxy-5,8-dimethyl-6-(2-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3-c]isoquinoline-2-carbonitrile (7): It was synthesized by reaction of **3a** with chloroacetoniitrile. Yield: 93 %; m.p.:260-262 °C. IR: 3463 (O-H); 3332,3229 (N-H); 2961, 2915 (C-H, sp^2); 2202 (C≡N); 1709 (C=O, acetyl). ^{1}H NMR: δ, 7.82(t, *J* = 63.0, 98.3 Hz, 1H,1 Ar-H),7.59 (t, *J* = 60.0, 58.3 Hz, 1H, 1Ar-H), 7.49(d, *J* = 60.0, 68.3 Hz, 1H, 1Ar-H), 7.15 (d, *J* = 61.0, 48.3 Hz, 1H,1 Ar-H); 6.52(s, 2H, CH_2), 5.21 (d, *J* =38.8 Hz, 1H, OH), 4.74(s, 1H, C^8H), 3.55 (d, *J* =16.0 Hz, 1H, C^5H), 3.35(d, 1H, C^7H),3.14(d, *J* =17.5 Hz, 1H, C^5H); 2.07 (d, 6H, $COCH_3$, CH_3);1.32 (s, 3H, CH_3).

^{13}C NMR (101 MHz, DMSO) δ 209.67 ,158.96, 158.37, 152.52, 149.51, 144.26, 137.83, 133.86, 131.71 ,128.81, 128.38, 125.24, 121.49, 116.22, 73.89, 67.82, 64.80, 41.95, 31.83, 28.40, 24.15.

Anal. calcd for **$C_{23}H_{20}N_4O_5S$**(464.1): C, 59.47; H, 4.34; N, 12.06. Found C, 59.71; H, 4.22; N, 12.21 %**.**

3.2.7. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(2-nitrophenyl)-5,6,7,8-tetra-hydroisoquinolin-3-yl)thio]-*N*-phenylacetamide (9a). It was synthesized by reaction of **2** with *N*-phenyl-2-chloroacetamide (**9a**). Yield: 96%; m. p.: 183-186 °C. IR: 3476 (O-H); 3308, 3139 (N-H); 2970, 2918 (C-H, sp^2); 2216 (C≡N); 1687(C=O, acetyl).

^{1}H NMR (400 MHz, DMSO) δ 10.19 (s, 1H, NH), 7.85 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.55 (m, 4H, 4Ar-H), 7.25 (m, 3H, 3Ar-H), 7.11 (d, *J* = 7.3 Hz, 1H, Ar-H), 5.12 (d, *J* = 9.6 Hz, 1H, OH), 4.94 (s, 1H, C^8H), 4.10 (m, 2H, SCH2), 3.17 (dd, *J* = 29.1, 13.5 Hz, 2H, C^5H, ^{C7}H), 2.91 (d, *J* = 17.2 Hz, 1H, C^5H), 1.98 (d, *J* = 29.0 Hz, 6H, COCH3, CH3), 1.30 (s, CH3, 3H).

^{13}C NMR (101 MHz, DMSO) δ208.28, 166.79, 160.51, 160.21, 160.00, 150.79, 149.93, 149.62, 139.09, 137.85, 134.12, 131.95 ,131.62, 129.61, 125.03, 119.99, 115.71 ,101.17,

72.42, 68.13, 67.94, 66.99, 65.07, 43.47, 40.50, 40.30, 40.09, 39.88, 39.67, 35.27, 31.82, 28.08, 24.06.

Anal. Calcd. for $C_{28}H_{26}N_4O_5S$ (530.16): C, 63.38; H, 4.94; N, 10.56 %. Found: C, 63.55; H, 5.00; N, 10.45%.

3.2.8. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(2-nitrophenyl)-5,6,7,8-tetra-hydroisoquinolin-3-yl)thio]-*N*-(4-tolyl)acetamide (**9b**). It was synthesized by reaction of **2** with *N*-(4-tolyl)-2-chloroacetamide (**9b**).Yield: 94%, m. p.: 209- 210°C. IR: IR: 3457 (O-H); 3305 (N-H); 3005, 2918(C-H, sp^2); 2215 (C≡N); 1693 (C=O, acetyl).

^{1}H NMR (400 MHz,) δ 10.10 (s, 1H, NH), 7.85 (d, *J* = 8.2 Hz, 1H,Ar-H), 7.59 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.50 (t, *J* = 7.6 Hz, 1H, Ar-H), 7.40 (d, *J* = 8.4 Hz, 2H, 2Ar-H), 7.19 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.08 (d, *J* = 8.3 Hz, 2H, 2Ar-H), 5.12 (d, *J* = 9.6 Hz, 1H,OH), 4.94 (s, 1H, C^8H), 4.17 – 4.05 (m, 2H, SCH_2), 3.17 (dd, *J* = 28.9, 13.4 Hz, 2H, C^5H, C^7H), 2.90 (d, *J* = 17.2 Hz, 1H, C^5H), 2.24 (s, 3H, CH_3), 2.00 (d, *J* = 29.1 Hz, 6H $COCH_3$, CH_3), 1.30 (s, 3H, CH_3).

^{13}C NMR (101 MHz, DMSO) δ 209.22, 166.20, 160.50, 158.29, 137.35, 136.86, 134.13, 132.77, 131.60, 129.56, 128.96, 128.59, 125.29, 119.64, 115.41, 104.48, 68.13, 65.04, 56.51, 43.47, 35.22, 31.84, 28.06, 24.07, 20.86.

Anal. Calcd. Anal. calcd for $C_{29}H_{28}N_4O_5S$ (544.18): C, 63.95; H, 5.18; N, 10.29 %. Found: C, 64.12; H, 5.21; N, 10.19 %.

3.2.9. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(2-nitrophenyl)-5,6,7,8-tetra-hydroisoquinolin-3-yl)thio]-*N*-(4-acetylphenyl)acetamide (9c): It was synthesized by reaction of **2** with *N*-(4-acetylphenyl)-2-chloroacetamide (**9c**). Yield: 96 %; m. p.: 210-212°C: IR: 3447 (O-H); 3267 (N-H); 3005, 2973(C-H, sp^2); 22156 (C≡N); 1685 (C=O, acetyl). ^{1}H NMR (400 MHz, DMSO) δ 10.55 (s, 1H, NH), 7.87 (dd, *J* = 22.1, 8.2 Hz, 3H,Ar), 7.67 (d, *J* = 8.3 Hz, 2H, 2Ar-H), 7.58 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.49 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.18 (d, *J* = 7.8 Hz, 1H, Ar-H), 5.11 (d, *J* = 9.7 Hz, 1H, OH), 4.94 (s, 1H, C^8H), 4.21 (m, 2H, SCH_2), 3.27 (dd, *J* = 28.3, 13.5 Hz, 2H, C^5H, C^7H), 2.91 (d, *J* = 17.3 Hz, 1H, C^5H), 2.51 (s, 4H, C7, CH_3), 2.04 (s, 3H,CO CH_3), 1.93 (s, 3H, CH_3), 1.30 (s,

3H,CH_3). ^{13}C NMR (101 MHz, DMSO) δ 209.72 ,167.54, 167.41, 160.52, 158.09, 150.97, 149.25, 143.55, 137.10, 134.14, 132.30, 131.50, 129.93, 129.00, 125.25, 118.90, 115.37, 104.38, 68.16, 64.69, 43.45,35.27, 33.27, 32.11, 27.88, 26.75, 23.93.

Anal. Calcd. for $C_{30}H_{28}N_4O_6S$ (572.17): C, 62.92; H, 4.93; N, 9.78%. Found: C, 62.80; H, 5.12; N, 9.89 %.

3.2.10. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(2-nitrophenyl)-5,6,7,8-tetra-hydroisoquinolin-3-yl)thio]-*N*-(4-chlorophenyl)acetamide (9d): It was synthesized by reaction of **2** with *N*-(4-chlorophenyl)-2-chloroacetamide (**9d**). Yield: 92 %; m.p.: 202-204°C. IR: 3539 (O-H); 3218 (1H, N-H); 3029, 2976 (C-H, sp^2); 2209 (C≡N); 1716,1697 (C=O, acetyl) . ^{1}HNMR : δ (400 MHz) δ 10.34 (s, 1H, NH), 7.85 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.65 – 7.44 (m, 4H, 4Ar-H), 7.32 (d, *J* = 8.8 Hz, 2H, 2Ar-H), 7.18 (d, *J* = 7.8 Hz, 1H, Ar-H), 5.11 (d, *J* = 9.8 Hz, 1H, OH), 4.94 (s, 1H, C^8H), 4.13 (d, *J* = 5.2 Hz, 2H, SCH_2), 3.19 (dd, *J* = 26.8, 13.5 Hz, 2H, C^5H, C^7H), 2.90 (d, *J* = 17.4 Hz, 1H, C^5H), 2.04 (s, 3H, CH3), 1.95 (s, 3H, CO CH3), 1.30 (s, 3H, CH3). ^{13}CNMR : δ (101 MHz, DMSO) δ 209.30, 166.74, 160.49, 158.18, 151.04, 149.28, 138.31, 137.30, 134.15, 131.58, 129.13 ,128.9, 127.36, 125.31, 121.07, 115.43, 104.38, 68.09, 64.98, 56.51, 43.48, 35.22, 31.89, 27.99, 24.07.20.01. Anal. Calcd. For $C_{28}H_{25}ClN_4O_5S$ (564.12) : C, 59.52; H, 4.46; N, 9.92%. Found : C, 59.63; H, 4.20; N, 9.99%.

3.2.11. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(2-nitrophenyl)-5,6,7,8-tetra-hydroisoquinolin-3-yl)thio]-*N*-(4-methoy- phenyl)acetamide (9e): It was synthesized by reaction of **2** with *N*-(4-methoxyphenyl)-2-chloroacetamide (**9e**). Yield: 89 %; m.p.: 195-197 °C. IR: 3583 (O-H); 3303 (N-H); 2998-2944 (C-H, sp^2); 2213 (C≡N); 1698 (C=O, acetyl). ^{1}H NMR (400 MHz, DMSO) δ 10.05 (s, 1H, NH), 7.86 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.65 – 7.36 (m, 4H, 4 Ar-H), 7.19 (d, *J* = 7.8 Hz, 1H, Ar-H), 6.85 (d, *J* = 8.7 Hz, 2H, 2Ar-H), 5.12 (d, *J* = 9.6 Hz, 1H, OH), 4.94 (s, 1H, C^8H), 4.10 (d, *J* = 6.8 Hz, 2H, SCH_2), 3.71 (s, 3H, OCH_3), 3.18 (dd, *J* = 27.9, 13.5 Hz, 2H, C^7H, C^5H), 2.91 (d, *J* = 17.3 Hz, 1H, C^5H), 2.01 (d, *J* = 24.9 Hz, 6H, $COCH_3$, CH_3), 1.30 (s, 3H, CH_3).

^{13}C NMR (101 MHz, DMSO) δ 209.24, 165.96, 160.50, 158.30, 155.80, 150.98, 149.33, 137.34, 134.13, 132.50, 131.60, 128.97, 128.58, 125.28, 121.22, 115.41, 114.36, 104.49, 68.13, 65.03, 55.63,40.64, 35.13, 31.86, 28.06, 24.08.

Anal. Calcd. for $C_{29}H_{27}N_4O_6S$ (559.2): C, 62.24; H, 4.86; N, 10.01. Found: C, 62.38; H, 4.90; N, 9.89%.

4. Synthesis of 7-Acetyl-1-amino-2-(*N*-arylcarbamoyl)-5,8-dimethyl-8-hydroxy-6-(2-nitrophenyl, 3-nitrophenyl or 4-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3-*c*]isoquinolines 10a,d; general methods.

4.1. Method A)

To a suspension of **9a, d** (10 mmol) in abs. ethanol (60 mL), a methanolic sodium methoxide was added (prepared by dissolving 0.25 g of sodium in 30 ml of methanol). The reaction mixture was stirred for one hour at room temperature. The yellow solid that formed after dilution with water (60 ml) was collected, washed with water, dried in air and then recrystallized from dioxane to give **10a, d** respectively.

4.1.1. 7-Acetyl-1-amino-5,8-dimethyl-8-hydroxy-6-(2-nitrophenyl)-*N*-phenyl-6,7, 8,9-tetrahydrothieno[2,3-c]isoquinoline-*N*-phenyl-2-carboxamide (10a).

It was obtained by cyclization of compound **9a**. Yield: 93%; m. p.: 286-290°C. IR: 3585 (O-H); 3432 (N-H); 2925 (C-H, sp^2); 1697 (C=O, acetyl). ^{1}H NMR (400 MHz, DMSO) δ 9.40 (s, 1H, NH), 7.80 (d, *J* = 8.0 Hz, 1H, Ar-H),7.60 (m, 2H, 2Ar-H), 7.50 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.33 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.18 (d, *J* = 9.6 Hz, 2H, 2Ar-H), 7.1 (m, 4H, NH_2, 2 Ar-H), 5.21 (d, *J* = 6.8 Hz, 1H, OH), 4.78 (s, 1H, C^{6}H), 3.56 (dd, *J* = 27.9, 13.5 Hz, 2H, C^{9}H, C^{7}H), 3.1 6 (d, *J* = 17.3 Hz, 1H, C^{9}H), 2.07 (d, *J* = 28 Hz, 6H, $COCH_3$, CH_3), 1.34 (s, 3H, CH_3).

^{13}C NMR (100 MHz, DMSO) δ 166.86, 149.79, 128.85, 128.72, 122.02, 121.84, 108.92, 67.91, 40.79, 40.56, 40.34, 32.52, 32.21, 28.49, 28.35, 28.17, 27.86, 7.05.

Anal. Calcd. for $C_{28}H_{26}N_4O_5S$ (530.16): C, 63.38; H, 4.94; N, 10.56 %. Found: C, 63.57; H, 5.12; N, 10.22%.

4.1.2**. 7-Acetyl-1-amino-*N*-(4-chlorophenyl)-5,8-dimethyl-8-hydroxy-6-(2-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3-*c*]isoquinoline-2-carboxamide (10d)**. It was obtained by cyclization of compound **9d**. Yield:94 %; m. p.: 300-301°C. IR: 3450 (O-H); 2924 (C-H, sp^2); 1698 (C=O, acetyl). ^{1}H NMR (400 MHz, DMSO) δ 9.52 (s, 1H, NH), 7.83 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.74 (d, 2H, 2Ar-H), 7.59 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.50 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.38 (d, *J* = 9.6 Hz, 2H, 2Ar-H), 7.15 (m, 3H, NH_2, Ar-H), 5.21 (s, 1H, OH), 4.78 (s, 1H, C^6H), 3.50 (dd, *J* = 25, 27 Hz, 2H, C^9H, C^7H), 3.2 (s, 1H, C^9H), 2.09 (d, *J* = 28 Hz, 6H, $COCH_3$, CH_3), 1.34 (s, 3H, CH_3).
Anal. Calcd. for $C_{28}H_{25}ClN_4O_5S$ (564.12): C, 59.52; H, 4.46; N, 9.92%. Found: C, 60.03; H, 4.32; N, 9.84%.

4.2. Method B).

To mixture of compound **3a-c** (10 mmol) and respective *N*-aryl-2-chloroacetamide **9a-e** (10 mmol) in ethanol (60 mL) a methanolic sodium methoxide (1.30 g) was added. The resulting mixture was refluxed for 110 mins hours. The solid that formed while hot was recrystallized from dioxane to give compounds **10a, d**; yield: 80-86%.

5. 1 Cytotoxicity against human cancer cell lines

In this work, all produced compounds anticancer activity were assessed for IC_{50} against two cell lines **HEPG2** and **HCT116** cells were examined using the sulphorhodamine-B (SRB) test. Cells were seeded at a density of 3 *103 cells/well in 96-well microtiterplates [34]. They were allowed to connect for 24 hours before incubating with our compounds. Cells were treated with various doses of our synthesized compounds (0, 12.5, 25, 50, and 100 µg/mL). For each concentration, three wells were employed, and the incubation period lasted 48 hours. DMSO was employed as the control vehicle (1% v/v). At the end of the incubation, the cells were fixed with 20% trichloroacetic acid and stained with 0.4% SRB dye. The optical density (O.D.) of each well was measured

spectrophotometrically at 570 nm with an ELISA microplate reader. The proportion of cell survival was determined as follows: Survival fraction = O.D. (treated cells) / O.D. (control cells). Sigmoidal dose-response curve-fitting models (GraphPad Prizm software, version 5) were used to obtain the IC_{50} (concentration that inhibits cell growth by 50%) for each chemical.

5.2. Cell cycle analysis:

The cell cycle arrests of compound **3** against **HEGP2** at their IC_{50} concentration was carried out according to Abcam method (code ab139418), (www.abcam.co.jp) . Thus, **HEPG2** cells were collected using 75% ice-cold ethanol and kept at -20 °C for 1 hour following treatment with an IC_{50} dose of our compounds **3.** The cells were then centrifuged, washed twice with ice-cold PBS, and incubated at 4°C for 20 minutes. The cell cycle was assessed using a flow cytometry kit (Propidium Iodide [ab13941]). Finally, use the Cell Quest software to undertake statistical analysis of the cell fractions in sub-G0/G1, S, and G2/M phases [35,36].

5.3. Annexin-V FITC apoptosis assay

The Annexin-V FITC apoptosis assay of compounds **3** against **HEPG2** at their IC_{50} values was performed according to (BioVision Research Products (code k101-25). (www.biovision.com).Thus, **HEPG2** cell line were treated with (6.4 µM) of the compound **3** for 24 h then collected by trypsin, centrifuged then rinsed with PBS and suspended in binding buffer, then dual-stained with Annexin V-FITC (5 µL) and propidium iodide (5 µL) in the dark for 15 min at romm temp. using flow cytometry to measure the cells with an excitation wavelength of 488 nm and an emission wavelength of 530 nm. The final results were analyzed with the Cell quest software [37, 38].

6. docking Materials and Methods

Molecular docking studies were performed in **(I Mole Lab for bioinformatics-Cairo).**

6.1. ligand Preparation:

The retrieved Ligands structures were subjected to energy minimization using the Avogadro 1.2.0 software with the MMFF94 force field [39].

6.2. Protein Target Selection and Preparation:

The selected target was RET tyrosine kinase receptor (UniProt ID: Q9UMQ4). The protein structures were prepared using AutoDock Tools 1.5.7 [40].

6.3. Binding Site Identification:

The potential binding pockets on the selected protein targets were identified using the CB-Dock 2 webserver [41].

6.4. Molecular Docking:

Molecular docking studies were performed between the energy-minimized phytochemicals and the prepared protein targets using AutoDock Vina (Eberhardt et al, 2021) to predict the binding affinities and investigate the intermolecular interactions [42].

6.5. Data Analysis and Visualization:

The results from molecular docking prediction were analyzed using appropriate computational tools and software. The visualization of protein-ligand interactions and the generation of figures were performed using.

7. Conclusion

This paper introduced a new series 5,6,7,8 tetrahydroisoquniolines and related tetrahydrothieno[2,3-c] isoquinolines based on 4-(2-nitrophenyl) moieties. These compounds were evaluated for their anticancer activity against two cell line **HEPG2** and **HCT116**, Moreover, the cell cycle arrest and apoptosis induction of compound **3** was studied, which caused cell cycle arrest of **HEPG2** cell line at G2/M phase and caused high increase in the early and late apoptosis and necrosis by 50%. Furthermore, the molecular docking of two compounds **3, 7** were studied they give good interaction with the RET enzyme. In the future we intend to synthesis more series of 5,6,7,8-

tetrahydrothieno[2,3-c] isoquinolines to studied there *in vitro* anticancer against several cell lines. Also to apply the *in vivo* anticancer study.

Authors' contributions

Abdelreheem A. Saddik: Investigation, Methodology, Writing–original draft, Visualization, Software, Validation. **Etify A. Bakhite**: Conceptualization, Formal analysis, Supervision, Investigation. **Reda Hassanien**: Investigation, Methodology. Writing–review & editing. **Nasser Farhan:** Writing–original draft, Writing–review & editing. **Eman M. Sayed**: Writing–review & editing, software, validation Conceptualization, Formal analysis, Investigation, Methodology. **Marwa Sharaky [3]**: Conceptualization, Formal analysis, Supervision, Investigation, Methodology, Writing–original draft, Writing–review & editing.

The corresponding authors emails

Etify A. Bakhite* – Department of Chemistry, Faculty of Science, Assiut University, Assiut 71516, Egypt; Orcid.org/0000-0003-3994-5629**;** Phone: +201006670292; Email: **etafy@aun.edu.eg**

Eman M. Sayed * – Chemistry Department, Faculty of Science, New Valley University, 72511 El-Kharja, *Egypt*, Phone: +201026662101, Email: **Emanmohsen@sci.nvu.edu.eg**

Authors

Abdelreheem A. Saddik- Department of Chemistry, Faculty of Science, Assiut University, Assiut 71516, Egypt**. Email:abdelreheem@aun.edu.eg**

Etify A. Bakhite* – Department of Chemistry, Faculty of Science, Assiut University, Assiut 71516, Egypt; Orcid.org/0000-0003-3994-5629**;** Phone: +201006670292; Email: **etafy@aun.edu.eg**

Reda Hassanien - Chemistry Department, Faculty of Science, New Valley University, 72511 El-Kharja, Email: **reda.h@sci.nvu.edu.eg**

Nasser Farhan – Chemistry Department, Faculty of Science, New Valley University, 72511 El-Kharja, *Egypt*. Email: **nasser-farhan@sci.nvu.edu.eg.**

Eman M. Sayed * – Chemistry Department, Faculty of Science, New Valley University, 72511 El-Kharja, *Egypt*, Phone: +201026662101, Email: **Emanmohsen@sci.nvu.edu.eg.**

Marwa Sharaky - Cancer biology department, national cancer institute, Cairo University , Egypt, **Email: marwa.sharaky@nci.cu.edu.eg.**

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available. It contains IR, ^{1}H NMR and ^{13}C NMR spectral data as well as the raw data of biological activity.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are in this published article and supplementary information.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial.

Acknowledgment

This research received no funds…

8. REFERENCES

1. Hawash, M. Highlights on Specific Biological Targets; Cyclin-Dependent Kinases, Epidermal Growth Factor Receptors, Ras Protein, and Cancer Stem Cells in Anticancer Drug Development. Drug Res. 6 (9),471-478(2019).

2. Rama, R. M., Priyamvada, B. Isoquinoline Alkaloids: Promising Natural Compounds for Targeting Angiogenesis and Metastasis in Colon Cancer. Onco Therapeutics. 9(2), 13 – 23(2022).

3. Xiaolin, L., Jiajun, H. , Wanjun, L., Ze, L.,Ying, X., Wenzhe, M. APTM, a Thiophene Heterocyclic Compound, Inhibits Human Colon Cancer HCT116 Cell Proliferation Through p53-Dependent Induction of Apoptosis. DNA Cell Biol. 37(2), 70–77(2018).

4. Rebecca, L. S., Nikita, S. W., Andrea, C., Robert, A. S., Ahmedin, J., Colorectal cancer statistics, A cancer journal for clinicians,73(3), 233-254 (2023).

5. Yi-Hao, Y., Kwong-Ming, K. Wei-, L., Yueh-Wei, L., Chih-Chi, W., Tsung-Hui , H., Ming-Chao, T., Yuan-Hung, K., Chih-Yun, L. Causes of Death among Patients withHepatocellular Carcinoma According to Chronic Liver Disease Etiology. Cancers (Basel). 15, 1687(2023).

6. Estes, C., Razavi, H., Loomba, R., Younossi, Z., Sanyal, A. J. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology. 67, 123–133 (2018).

7. Li, J., Zou, B., Yeo, Y. H., Feng, Y., Xie, X., Lee, D. H., Fuji, H., Wu, Y., Kam, L.Y., , Ji, F., et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999-2019: A systematic review and meta-analysis. Lancet Gastroenterol. Hepatol. 4, 389–398(2019).

8. Priyanka, M. , Vinit, R. , Archana, S., Ashok, K. S. , Amit, R., Pranesh, K. , Amit, K. K. , Arnab, D. , Amalesh, S., Umesh, K., Dinesh, K. , Biswanath, M. , Sneha, N. , Anand, P., Kausar, M., Sudipta, S. 6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid attenuates colon carcinogenesis via blockade of IL-6 mediated signal. Biomedicine & Pharmacotherapy. 100, 282-295(2018).

9. Liu-Jun, H ., Dong-Lin, Y., Shi-Qiang ,L. , Ya-Jun, Z., Yan, T., , Jie, L., Brendan, F., , Hui-Kuan, L. , Hong-Yu, L., Zhong-Zhu, C., Zhi-Gang, X. Facile construction of fused benzimidazole-isoquinolinones that induce cell-cycle arrest and apoptosis in colorectal cancer cells. Bioorg Med Chem. 26, 3899-3908(2018).

10. Madhavi, G., Suresh, E., Wang, Z. and Kinfe, K. R., Design and Synthesis of Tetrahydroisoquinoline Derivatives as Anti-Angiogenesis and Anti-Cancer Agents, Anti-Cancer Agents in Medicinal Chemistry, 21, 2505 – 2511 (2021).

11. Xuemei, D., Tian, L., Zhao, L., Huaixiu, W., Honghua, Z. , Xiaoyan, Y. , Fang, L., Dan, L., Tao, S . , Quanyi, Z . , Zhen, W., Design, synthesis and anti-hepatocellular carcinoma activity of 3-arylisoquinoline alkaloidsEuropean . J Med. Chem. 228, 113985 (2022).

12. Yuqing, W., Lin, L., Zhuo, L., Honghua, Z. Design, synthesis, and biological evaluation of 1-styrenyl isoquinoline derivatives for anti-hepatocellular carcinoma activity and effect on mitochondria. Eur J Med Chem. 256, 115420 (2023).

13. Galán A, Moreno L, Párraga J, Serrano A, Sanz M J, Cortes D, Cabedo NNovel isoquinoline derivatives as antimicrobial agents. Bioorg. Med. Chem. 21(11):3221−3230 (2013).

(4. Alagumuthu, M., Sathiyanarayanan, K. I., Arumugam, S. Molecular docking, design, synthesis, in vitro antioxidant and anti-inflammatory evaluations of new isoquinoline derivatives. Int. J. Pharm. Sci. 7 (12), 200−208(2015).

15. Manikandan, A., Sivakumar, A. A. Analgesic. anti-inflammatory and antipyretic evaluations of new isoquinoline derivatives. J. Pharm. Sci. 8 (4), 339−343(2016).

16. Watanuki, S., Matsuura, K., Tomura, Y., Okada, M., Okazaki, T., Ohta, M., Tsukamoto, S. Synthesis and pharmacological evaluation of 1-isopropyl-1,2,3,4-tetrahydroisoquinoline derivatives as novel antihypertensive agents. Chem Pharm Bull; 59 (8), 1029-1037(2011).

17. Pingaew, R., Prachayasittiku,l S., Ruchirawat, S. Synthesis, cytotoxic and antimalarial activities of benzoyl thiosemicarbazone analogs of isoquinoline and related compounds. Molecules 15 (2), 988-996(2010).

18. Hassaneen, H. M., Wardkhan, W. W., Mohammed, Y. S., Novel route to isoquinoline[2,1-g] [1,6] naphthyridine, pyrazolo[5,1-a]isoquinoline and pyridazino[4′,5′: 3,4]pyrazolo[5,1-a]isoquinoline derivatives with evaluation of antitumor activities. Z. Naturforsch. B. 68 (b), 895−904(2013).

19. Kakhki, S., Shahosseini, S., Zarghi, A. Design, synthesis and cytotoxicity evaluation of new 2-aryl-5,6-dihydropyrrolo[2,1-a] isoquinoline derivatives as topoisomerase inhibitors. Iran. J. Pharm. Res. 13,71−77(2014).

20. Partik, Y., Ashish, K., Islam, A., Vishal, N. Recent development of tetrahydroquinoline /isoquinoline based compounds as anticancer agents. Med. Chem. 21 (17), 1587-1622 (2021).

21. Cushman, M., Jayaraman, M., Vroman, J. A., FuKunaga, A. K., Fox, B. M., Kohlhagen, G., Strumberg, D., Pommier, Y. Synthesis of new indeno[1,2-

c]isoquinolines:cytotoxic non-camptothecin to poisomerase 1 inhibitors. J. Med. Chem. 43 (20), 3688-3698 (2000).

22. Rashad, A. S., Ibrahim, A., Mohmed, M. Cytotoxcicity evaluation of a new set of2-aminobeno[de]isoquoinoline- 1,3-diones. Int. J. Mol. Sci. 15 (12), 22483-22491(2014).

23. Gao, Y., Tu, N., Liu, X., Lu, K., Chen, S., Guo, J. Progress in the Total Synthesis of Antitumor Tetrahydroisoquinoline Alkaloids. chemistry biodiversity 20 , e202300172. (2003).

24. Islam, S. M., Raed, M. M., Safia, S., Etify, A. B.,Walid, S. Thieno[2,3-c]isoquinolines: A novel chemotype of antiproliferative agents inducing cellular apoptosis while targeting the G2/M phase and Tubulin. Drug Dev. Res. 84(4), 747-766 (2023).

25. Faheem, K. K., Chandra, S, Chander S, Kunjiappan S, Murugesan S. Medicinal chemistry perspective of 1,2,3,4-tetrahydroisoquinoline analogs biological activities and SAR studies. RSC advances. 11(20), 12254-12287(2021).

26. Kisel, V. M., Kostyrko, E. O., Kovtunenko, V.A. Synthesis and biological properties of isoquinolines spirofused with carbocycles and heterocycles at position 4. *Chem. Heterocycl. Compd. 38*, 295-1318(2002).

27. Potikha, L. M., Kovtunenko, V.A. Synthesis and properties of 3-aminodihydro-isoquinolines. Russ. Chem. Rev. 78, 513-533(2009).

28. Ju, K. S., Parales, R. E. Nitroaromatic compounds, from synthesis to biodegradation. *Microbiol. Mol. Biol. Rev. 74*, 250-272(2010).

29. Noboru, O. The Nitro Group in Organic Synthesis; Wiley VCH: Weinheim, Germany1−Book, 363(2001).

30. Strauss, M. The nitroaromatic group in drug design. Ind. Eng. Chem. Prod. Res. Dev. 18, 158-166(1979).

31. Nepali, K., Lee, H.Y., Liou, J. P. Nitro-Group-Containing Drugs. *J. Med. Chem.* 62, 2851-2893(2019).

32. Eman, M. S., Reda, H., Nasser F., Hanan, F. A, Khaled, M., Shaaban, K. M., Joel, T. M., Etify, A. B. Nitrophenyl-group-containing heterocycles. Part I. Synthesis, characterization, anticancer activity and antioxidant properties of some new 5,6,7,8-tetrahydro-isoquinolines bearing 3(4)-nitrophenyl group. ASC Omega 7(1), 8767 – 8776 (2022).

33. Eman, M. S., Etify, A. B., Reda, H., Nasser, F., Hanan, F. A., Salma, G. M., Nivin, A. H. Novel tetrahydroisoquinolines as DHFR and CDK2 inhibitors: Synthesis, characterization, anticancer activity and antioxidant properties, BMC Chemistry 18(1): 34. (2024).

34. Tarfah, A., Ahmed, A. A., Ayman, A. E., Maha, A. A., Marwa, A., Taghreed, A. M., Medhat, A., Ahmed, N., Wagdy, M. E., Marwa, S. Biological evaluation, docking studies, and *in silico* ADME prediction of some pyrimidine and pyridine derivatives as potential EGFRWT and EGFRT790M inhibitors. J. Enzyme Inhib. Med. Chem. 38(1), 176–191(2023).

35. Lili, X., Guozheng, H., Zhihui, Z., Shasha, T., Yingying, W., Huanwu, H., Xiaowei, L., Ying, L., Feize, L., Huajun, Z. LFZ-4-46, a tetrahydroisoquinoline derivative, induces apoptosis and cell cycle arrest via induction of DNA damage and activation of MAPKs pathway in cancer cells. Anticancer Drugs 32 (8), 842-854(2021).

36. Megda, F. M., Hamdi, M. H., Ismail, A. A. Cytotoxicity, molecular modeling, cellcycle arrest and apoptotic induction induced by novel tetrahdro [1,2,4]triazolo[3,4-a] isoquinoline chalcones. Eur. J. Med. Chem.143, 532-541(2018).

37. Mohammed, F. Z., Rizzk, Y. W., El Deen, I. M., Mourad, A. A. E., El Behery, M. Design, Synthesis, Cytotoxic Screening and Molecular Docking Studies of Novel Hybrid Thiosemicarbazone Derivatives as Anticancer Agents. Chemistry & Biodiversity. 18, e2100580 (2021).

38. Mohammed, F. Z., Rizzk, Y. W., MoheyEl-Deen, I., Gad, E. M., El Behery, M., Mahdy, A. R. E. Discoveryof 2-Amino-4H-1,3, 4-thiadiazine-5(6H)-oneDerivativesand TheirIn Vitro AntitumorInvestigation. MedicinalChemistry& DrugDiscovery. 7, 246962242 (2022).

39. Marcus, D. H., Donald, E. C., David, C. L., Tim V., Eva, Z. and Geoffrey, R. H., "Avogadro: An advanced semantic chemical editor, visualization, and analysis platform" Journal of Cheminformatics 4, (2012). DOI:10.1186/1758-2946-4-17

40. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J., Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. J. Computational Chemistry, 16: 2785-91 (2009).

41. Liu, Y., Yang, X., Gan, J., Chen, S., Xiao, Z. X., Cao, Y., CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic acids research, 50, 159–164 (2022).

42. Eberhardt, J., Santos-Martins, D., Tillack, A. F., Forli, S., AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. Journal of Chemical Information and Modeling,61, 3891-3898 (2021).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementryInformation.docx