SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL PYRAZOLO[3,4-D]PYRIMIDINE DERIVATIVES OF EXPECTED ANTI-CANCER ACTIVITY

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ABSTRACT

A series of novel pyrazolo[3,4-d]pyrimidine derivatives have been designed and synthesized in synthetically useful yields. All the new synthesized compounds were biologically evaluated in vitro for their cytotoxic activities against a panel of three cancer cell lines namely, HepG-2, MCF-7, and HCT-116. The results of cytotoxic evaluation indicated that compound (9) exhibited the most prominent cytotoxic effect against all tested cell lines with IC₅₀ values ranging from (4.03-6.18) µM comparable to that of doxorubicin as a control drug (IC₅₀ values of 8.17 and 9.27 µM). In particular, compounds (8,9,11) and (12) exhibited higher intercalative activity with IC₅₀ value of 30 µM than doxorubicin (31 µM).

Keywords: Anticancer, pyrazolopyrimidine derivatives, Topoisomerase II, DNA-intercalator.
I-INTRODUCTION:

Cancer remains one of the most common causes of death throughout the world and thus the development of potent and more effective anticancer agents represents one of the most important challenges in therapeutics due to the unrivaled pathophysiology of tumors and the predictable emergence of resistance to medication (Thun et al. 2010). Classical methods for cancer treatment including radiotherapy, chemotherapy, and immunotherapy with their own limitations.

Anticancer drugs have been classified into two main target types: the first one is drugs that target DNA, RNA, or proteins. The second target includes other elements involved in the carcinogenesis process, such as the immune system, the endothelium and the extracellular matrix. Most classical chemotherapeutic agents interact with tumor DNA (Espinosa et al. 2003). Compounds that affect DNA include groove binders, alkylating agents, DNA intercalators, and topoisomerase inhibitors (Hurley 2002).

Topoisomerases are important nuclear enzymes, which play a pivotal role in DNA replication, transcription, chromosome segregation, and recombination. There are two fundamental types of topoisomerases; (a) topoisomerase I (Topo I), which is responsible for cleavage, relaxing, and releasing of one strand of the DNA duplex, (b) topoisomerase II (Topo II), which cleaves both strands of the DNA helix simultaneously to remove DNA super coiling (Wang 2002). These enzymes covalently bind to DNA helix via tyrosine residues in the active site. These linkages are transient and easily reversible, and the covalently bound structure is known as the cleavable complex (Denny 2004). Accordingly, topoisomerases are considered as crucial targets for cancer chemotherapy treatments (Pommier et al. 2010). Topoisomerase inhibitors block the ligation step of the cell-cycle, generating single and double stranded breaks that harm the integrity of the genome (Mlcochova et al. 2018). Introduction of these breaks subsequently leads to apoptosis (Kaina2003).

Some anticancer drugs targeting Topo II inhibit the enzymatic activity as a primary mode of action and are known as catalytic Topo II inhibitors (Nitiss 2009). Another type of Topo II-targeting drugs, including intercalating drugs, interfere with the enzyme’s cleavage and rejoining activities by trapping the cleavable complex and thereby increasing the time of the transient Topo II-catalyzed DNA breaks. These drugs are referred to as Topo II poisons because they convert the Topo II enzyme into a DNA-damaging agent (Pommier et al. 2010, Nitiss 2009).

These class of drugs act either by topo poisoning via inter-chelation with DNA as doxorubicin (Liu et al. 1989), a msacrine (Sung et al. 2005) and mitoxantrone (Shenkenberg et al. 1986). On the other hand, drugs act as catalytic inhibitors of Top-II as TSC24 (Huang et al. 2010), HY-1 (zhao et al. 2011) and compound (I) (Islam et al. 2017).

DNA Intercalators and Topo II poisons share three common essential structural features. The first one is a planar polyaromatic system (chromophore) which is sandwiched between DNA base-pairs (Laponogov et al. 2013). The second feature is a cationic species, interacting with the negatively charged phosphate group of DNAs. The
cationic center may be an amino or nitrogen containing heterocyclic group, which can be protonated at physiological pH (Lee et al. 2017). The third feature is a flexible side chain that anchors DNA (Bailly et al. 2012) (Figure1).

On the other hand, pyrazolopyrimidine moieties have anticancer activities (Schenone et al. 2014). In addition, the discovery of new therapeutic DNA intercalators for the treatment of cancer are considered one of the most important goals in the field of medicinal chemistry (Varrica et al. 2018). Pyrazolopyrimidine analogs exhibited excellent anticancer activities through DNA intercalation (Cheng and Robins, 1956) (El-Enany et al., 2010). Pogorelčnik and co-workers optimized the first anti-topoisomerase II pharmacophore belonging to pyrazolo[3,4- d]pyrimidine scaffold performing systematic screening to predict the bioactivity between molecule and drug target. Compound (II) was a result of this high-throughput screening (HTS) and efficacious candidate in the series of pyrazolo[3,4- d]pyrimidine which showing promising anticancer activities in hepatocellular carcinoma (HepG2) and breast cancer (MCF-7) cell lines with mean IC₅₀ value of 1.30 μM. besides, its topoisomerase inhibition (Pogorelčnik et al. 2015) (Figure2).

(Figure 1). DNA intercalators and their basic pharmacophoric features
Compound (III) proved to be the most active and efficacious candidate in this series, with mean IC\textsubscript{50} values of 1.30 μM. Further biological evaluation suggested that this compound induce apoptosis and inhibit human topoisomerase (Topo) IIα (Singla et al. 2017). On the other hand, roscovitine, belongs to the family of purine and is used for the treatment of lung cancer with IC\textsubscript{50} value of 2.7 μM (Whittaker et al. 2004).

Therefore, on the basis of previously mentioned findings and in resumption of our previous efforts in the design and synthesis of new anticancer agents (Gaber et al. 2018), we report the design, synthesis and anticancer activities of new pyrazolo[3,4-d]pyrimidine derivatives. These derivatives were designed based on the main pharmacophoric features of DNA intercalators.

II. Results and Discussion:

II.1 Chemistry:

The designed compounds were synthesized as outlined in schemes 1 and 2. Ethoxymethylene malononitrile (1) (Ding et al. 2012) was allowed to reflux with commercially available phenyl hydrazine in ethanol to produce 5-amino-1-phenyl-1H-pyrazole-4-carbonitrile (2) (Cheng et al. 1956). Compound (2) underwent a partial hydrolysis using alcoholic sodium hydroxide to produce carboxamide derivative (3) (He et al. 2011). 6-methyle-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidine core (4) (Miyashita et al. 1990) was formed from the reaction of compound (3) with ethylacetate, sodium ethoxide in absolute alcohol with subsequent chlorination using phosphoryl trichloride to afford compound (5) (Miyashita et al. 1998). The obtained compound (5) was refluxed with hydrazine hydrate to afford 4-hydrazinyl-6-methyl-1-phenyl-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidine (6) (Gaber et al. 2018). Compound (4) using phosphorous pentasulfide in pepridine also we used lawesson reagent in THF afforded unappropriated yield (9)(Elsebaei et al., 2019; Mancy et al., 2019) so that the \textsuperscript{1}H-NMR...
spectrum of compound (6) revealed singlet signal at 14.32 ppm corresponding to (NH$_2$)-proton and compound (6) singlet signal at 13.65 ppm corresponding to (NH)proton. Furthermore, compounds (7) and (8) were prepared by cyclo-condensation of compounds (6) with 2,6-dichlorobenzaldehyde and cinnamaldehyde in absolute ethanol with catalytic amount of glacial acetic acid to give the corresponding hydrazones (7) and (8) respectively.

![Scheme 1](image)

Thiole derivative (9) was synthesized from compound (4) through reflux with phosphorous pentasulfide in piperidine for 6 hours. The structure of compound (9) was confirmed by different analytical techniques as IR and H$^1$ NMR. The H$^1$ NMR spectrum of compound (9) revealed singlet signal at 14.32 ppm corresponding to (-SH)-proton.

For preparation of alkyl derivatives (10-13) were prepared by reaction of appropriate alkyl bromides, namely 1-bromoisobutan, 1-bromononane, 1-bromo-3-methylpentane, 1-bromo-2-ethylbutane with compound (9) in the presence of anhydrous potassium carbonate in DMF. This reaction proceeded smoothly and the desired compounds were obtained in good yields (68-75%).(El-Gamal et al., 2015)
II.2. Experimental.

II.2.1. General:

Melting points were measured on a Gallen-kamp melting point apparatus and were uncorrected. The IR spectra were recorded on Nikolet IR 200 FT IR spectrophotometer using KBr discs (λ max in cm⁻¹). H¹ NMR and C¹³ NMR spectra were performed on Gemini 300BB spectrometer at 300MHz and Bruker spectrometer at 75MHz, respectively. TMS was used as internal standard and DMSO-d₆ as solvent. The chemical shifts were reported in ppm (δ) and coupling constant (J) values were given in Hertz (Hz). Signal multiplicities were represented by s (singlet), d (doublet), t (triplet), q (quadruplet), and m (multiplet). All of the new compounds were analyzed for C, H and N and agreed with the proposed structures within±0.4% of the theoretical values by the automated CHN analyzer. Mass spectra were recorded on a unit of Shimadzu GCMS-QP/MS-QP5050A spectrometer operating at 70 ev. The purity of the compounds was checked by thin layer chromatography (TLC) using Merck silica gel 60 F254 recoated sheets.

**General procedure for preparation of compounds (7) and (8).**

Anmixture of hydrazine derivative (6) (0.30 g, 0.001 mol), 2,6-dichlorobenzaldehyde, cinnamaldehyde (0.001 mol) and catalytic amount of glacial acetic acid (0.5 ml) was heated under reflux in absolute ethanol (20 ml) for specific time. The precipitate that formed on hot was filtered and crystallized from ethanol to yield the title compounds (7) and (8).
4-(2-(2,6-Dichlorobenzylidene) hydrazinyl)-6-methyl-1-phenyl-1H-pyrazolo[3,4-d]/pyrimidine (7)

Red solid; reaction time: 6 h; yield 83% (0.38 g); m.p.: 255-257°C; IR (KBr, ν, cm⁻¹), 3383(NH), 3109 (Ar-H), 2910(aliph-CH).¹HNMR(DMSO-d₆); 8.55 (s, 1H, NH, H4), 8.39 (s, 1H, phenyl-H), 8.24 (d, 2H, J = 7.3 Hz, phenyl-H2, H6), 8.18 (s, 1H- pyrazol), 7.60-7.65 (m, 3H, phenyl-H3, H4, H5). 7.39 (d, 2H, J = 7.3 Hz, phenyl-H2, H6). 7.38 (m, 1H, phenyl-H5) , 2.33 (s, 3H, pyrimidine- CH₃).¹³C NMR (DMSO, d₆): 165.9, 156.1, 154.8, 141.4, 139.1, 136.6, 134.1, 131.1, 131.6, 130.2, 129.9, 127.1, 126.8, 125.2, 99.4, 25.9. MS (m/z): 398.28 (M⁺, 76.45%), 396.33 (M⁺², 100%); Anal. Calcd. for C₁₉H₁₄Cl₂N₆ (397.26): C, 57.45; H, 3.55; N, 21.16. Found: C, 57.42; H, 3.51; N, 21.21.

6-Methyl-1-phenyl-4-(2-((1E,2E)-3-phenylallidene) hydrazinyl)-1H-pyrazolo[3,4-d]/pyrimidine (8)

Orange needle, reaction time: 5 h; yield 95% (0.39 g); m.p.: 241- 243°C; IR (KBr, ν, cm⁻¹), 3317(NH), 3112 (Ar-H), 3009(aliph-H).¹HNMR (DMSO-d₆); 10.46 (s, 1H, NH, H4), 8.39 (d, 2H, J = 7.4 Hz, phenyl-H2,H6), 8.18 (s, 1H-pyrazol), 7.94 (s, 1H, N=CH), 7.52-7.60 (m, 3H, phenyl-H3, H4, H5). 7.54 (d, 2H, J = 7.4 Hz, phenyl-H2, H6), 7.33-7.45 (m, 3H, phenyl-H3, H4, H5). 7.22 (d, 1H, J =7.5 Hz , CH=CH-ph), 6.89 (d, 1H, J = 7.5 Hz, N=CH=CH=CH).¹³C NMR (DMSO-d₆): 164.2, 155.3, 154.5, 149.3, 139.2, 139.1, 137.4, 136.4, 129.8, 129.6, 127.6, 126.9, 125.1, 122.5, 99.5, 56.5, 25.5, 18.9. MS (m/z): 354.42 (M⁺, 100%), 350.96 (3.64%). Anal. Calcd. for C₂₁H₁₈N₆ (354.42): C, 71.17; H, 5.12; N, 23.71. Found: C, 71.14; H, 5.18; N, 23.74.

6-Methyl-1-phenyl-1H-pyrazolo[3,4-d]/pyrimidine-4-thiol (9)

Phosphorus pentasulfide (1.67 g, 3.75 mmol) in DMF. This reaction proceed smoothly and the desired compound DMF (20 ml) was heated under reflux for 10 hours then poured onto ice-cold cold. Another white compound was filtered off and recrystallized in petroleum ether to afford compound (9).

Yellow crystal; yield 91% (0.28 g); m.p. 230-232°C. IR (KBr, ν, cm⁻¹), 3009 (C-H aromatic), 2933 (C-H aliphatic), 1498 (C=N).¹HNMR(DMSO-d₆), 13.15 (s, 1H, SH D₂O exchangeable), 8.20 (d, 2H, J = 7.5 Hz, phenyl-H2, H6), 8.16 (s,1H, pyrazol),7.52-7.60 (m, 3H, phenyl-H3, H4, H5). 2.37 (s, 3H, pyrimidine-CH₃).¹³C- NMR (DMSO-d₆): 164.7, 158.2, 138.2, 133.3, 129.3, 126.9, 119.9, 104.9, 30.5, 24.1. MS (m/z): 242.30 (M⁺, 75.74%), 241.62 (100%). Anal. Calcd. For C₁₉H₁₈N₆S (242.30): C, 59.48; H, 4.16; N, 23.12. Found: C, 59.53; H, 4.19; N, 23.16.

General procedure:

Analogously to the previous scheme, compounds (10-13) were prepared by the reaction of alkyl bromide with compound (9) in the presence of anhydrous potassium carbonate (0.001mol) in DMF. This reaction proceed smoothly and the desired compound DMF (20 ml) was heated under reflux for 10 hours then poured onto ice-cold
water. The obtained precipitate was filtered, washed with water, dried and crystallized from ethanol to afford compounds (10-13), respectively.

4-(Isobutylthio)-6-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (10)

Yellish crystals, (2.2 g: 76%); m.p. 80-83°C; IR (KBr)cm⁻¹ : 3017 (C-H aromatic), 2957 (C-H aliphatic), 1563 (C=N). ¹H-NMR (DMSO-d₆) δ ppm: 8.43 (d, 2H, J = 7.4 Hz, phenyl-H2, H6), 8.20 (s, 1H -pyrazol), 7.61-7.57 (m, 3H, phenyl-H3 H4 H5), 4.12 (d, 2H, J = 7.4 Hz, S-CH₂), 2.51 (s, 3H, pyrimidine-CH₃), 1.05 (m, 1H, CH-(CH₃)₂), 0.91 (d, 6H, J = 6.8 Hz, CH-(CH₃)₂). ¹³C NMR (DMSO-d₆): 166.2, 163.6, 155.5, 139.0, 133.3, 129.7, 127.0, 121.3, 101.9, 54.5, 40.5, 39.8, 28.5, 26.1. MS(m/z): 298.41 (51.53%, M⁺), 155.24 (100%). Anal. Calc. for (C₁₆H₁₈N₄S): C, 64.40; H, 6.08; N, 18.78; Found: C, 64.45; H, 6.12; N, 18.90.

Methyl-4-(nonylthio)-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (11)

Yellow crystals, (2.20 g: 70%); m.p. 85-95°C; IR (KBr)cm⁻¹: 3072 (C-H aromatic), 2925 (C-H aliphatic), 1559 (C=N). ¹H-NMR (DMSO-d₆) δ ppm: 8.46 (d, 2H, J = 7.4 Hz, phenyl-H2, H6), 8.17 (s, 1H -pyrazol), 7.37-7.60 (m, 3H-phenyl-H3 H4 H5), 3.37 (t, 2H, J=7.4 Hz, S-CH₂), 2.49 (s, 3H, pyrimidine-CH₃), 125.1-1.77(m, 6H, (CH₂)₃), 1.23-1.45 (m, 6H-(CH₂)₃), 0.86 (t, 3H, J = 7.2 Hz). ¹³C NMR (DMSO-d₆): 165.1, 164.8, 152.1, 152.1, 138.8, 129.7, 127.1, 121.5, 111.5, 40.5, 40.3, 39.7, 31.7, 29.2, 29.0, 28.8, 26.63, 22.54, 14.38. MS(m/z): 368.54 (21.52%, M⁺), 241.77(100%). (Anal. Calc. for (C₂₁H₂₈N₄S): C, 68.44; H, 7.66; N, 15.20; Found:C,68.41; H, 7.69; N, 15.24.

4-((2-Ethylbutylthio)-6-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (12)

Brownish crystals,(2.20 g: 72%); m.p. 85-90°C; IR (KBr)cm⁻¹: 3072 (C-H aromatic), 2962 (C-H aliphatic),1560 (C=N). ¹H-NMR (DMSO-d₆) δ ppm: 8.44 (d, 2H, J=7.4 Hz, phenyl-H2, H6), 8.16 (s, 1H -pyrazol), 7.36-7.59 (m, 3H-phenyl-H3 H4 H5), 3.37 (d, 2H- J =7.3 Hz, S-CH₂), 2.51 (s, 3H, pyrimidine-CH₃),1.60 (m,5H, 2CH₃), 0.93 (t, 6H, J =7.5 Hz , 2CH₃),¹³C NMR (DMSO-d₆): 165.1, 164.7, 152.1, 138.8, 133.6, 129.6, 127.1, 121.4, 111.5, 40.5, 40.3, 39.9, 39.7, 39.3, 31.7, 26.5, 25.3, 11.3; MS(m/z): 326.46 (24.90%, M⁺), 226 (100%). (Anal. Calc. for (C₁₈H₂₂N₄S): C, 66.22; H, 6.79; N, 17.16; Found:C,66.25; H, 6.83; N, 17.19.

4-(Isopentylthio)-6-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine (13)

Brownish crystals,(2.23 g: 75%); m.p. 80-87°C; IR (KBr)cm⁻¹: : 3076 (C-H aromatic), 2955 (C-H aliphatic),1550 (C=N). ¹H-NMR (DMSO-d₆) δ ppm: 8.47 (d, 2H, J = 7.4 Hz, phenyl-H2, H6), 8.18 (s, 1H -pyrazol), 7.61-7.59 (m, 3H, phenyl-H3 H4 H5), 3.41 (t, 2H, J = 7.4 Hz, S-CH₂), 2.51 (s, 3H, pyrimidine-CH₃), 1.74 (m, 2H, CH₂-CH-((CH₃)₂), 1.65 (m, 1H, CH-(CH₃)₂), 0.95 (d, 6H, J = 6.8 Hz, CH-(CH₃)₂).¹³C NMR (DMSO-d₆): 164.7, 152.3, 138.8, 133.5, 129.6, 127.0, 121.2, 111.4, 40.3, 39.9, 38.2, 27.4, 26.5, 22.5. MS(m/z): 312.44 (21.58%, M⁺), 263.31(100%). (Anal. Calc. for (C₁₇H₂₀N₄S): C, 65.35; H, 6.45; N, 17.93; Found:C,65.49; H, 6.50; N, 17.97.
4- Biological Evaluation:

4.1 *In Vitro* Anticancer Screening.

According to the rational drug design, a series of novel pyrazolopyrimidine derivatives were designed and synthesized. Consequently, the new synthesized compounds were evaluated for their *in vitro* cytotoxic activity against four different cancer cell lines namely, hepatocellular carcinoma (HepG-2), human breast adenocarcinoma (MCF-7) and colorectal carcinoma (HCT-116) via standard MTT method (Mosmann, 1983; Denizot and Lang, 1986; Thabrew *et al.*, 1997).

From the obtained anticancer results, it is evident that the screened compounds displayed different levels of cytotoxicity ranging from potent, moderate, weak, and inactive cytotoxicity against all tested cell lines. Therefore, data represented in (Table 1) revealed that, compound (9) was found to be significantly equipotent and efficient than doxorubicin with IC50 values of 4.50, 4.17 and 5.13μM against tested cell lines. Moreover, compound (9) was found to be the most potent derivatives against the three cell lines with IC50 values less than 30 μM compared with an anticancer drug, doxorubicin as control. Besides, compound (10) possessed moderate anti-proliferative activities against the three cell lines with IC50 values ranging from 18μM to 40μM. Furthermore, compound (13) showed weak anti-proliferative activities with IC50 values ranging from 75 to 96μM. Finally, compounds (7, 8, 11) and (12) appeared to be inactive against tested cell lines.

**Table 1:** *In-vitro* anti-proliferative activities towards HePG2, HCT-116 and MCF-7 cell lines.

| IC50 (μM) | HePG-2 | MCF-7 | HCT-116 |
|-----------|--------|-------|---------|
| **DOX**   | 4.50±0.2 | 4.17±0.2 | 5.23±0.3 |
| 7         | 100>   | 100>   | 100>    |
| 8         | 100>   | 100>   | 76.84±4.1 |
| 9         | 27.83±2.1 | 24.23±1.6 | 12.09±1.0 |
| 10        | 54.79±3.6 | 50.58±3.2 | 35.48±2.3 |
| 11        | 100>   | 100>   | 100>    |
| 12        | 100>   | 100>   | 100>    |
| 13        | 75.26±4.2 | 96.81±5.4 | 100>    |

Cytotoxic activity of some compounds against human tumor cells

For IC50 values of the active compounds are summarized in Table 1.
5-Conclusion:

A series of novel pyrazolo[3,4-d]pyrimidine derivatives have been designed and synthesized in useful yields. All the new synthesized compounds were biologically screened in vitro for their cytotoxic activities against a panel of four cancer cell lines namely, HepG-2, MCF-7 and HCT-116. The results of cytotoxic evaluation indicated that compound (6) was found to be significantly more potent and efficient than doxorubicin with IC₅₀ values of 4.50, 4.17 and 5.13 μM against tested cell lines. Moreover, Pharmacophoric features indicated that pyrazolo[3,4-d]pyrimidine scaffold having a four atoms linker was more potent than those possessing other linkers which lead to significant decrease in cytotoxic activity.

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**تشييد وتقييم بيولوجي لبعض مشتقات البيرازول (3,4-دي) بيريميدين الجديدة المتوقع لها نشاط مضاد للسرطان**

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في هذا البحث تم تصميم وتحضير بعض مشتقات البيرازول (3,4-دي) بيريميدين الجديدة من خلال بعض المركبات الوسيطة وقد تم تقييم النشاط المضاد للسرطان لهذه المركبات الجديدة في مقابل بعض أنواع السرطان كسرطان الثدي وسرطان الكبد وسرطان القولون وكذلك تم تقدير فائدتها هذه المركبات لمتكملات مع الحمض النووي وقد تم اختيار أنشطة هذه المشتقات لاختيار كميث أو تربويوميازاتان.

وتتم إثبات الصيد البيولوجي للأمراضية باستخدام جهاز الأشعة دون الحركة وجهاز الرنين النووي المغناطيسي ومطابقة الكلفة هذا بالإضافة إلى التحليل الدقيق لعناصر المركبات التي تم حساب الكربون والهيدروجين والتتراديغين في المركبات وتم أجراء الاختبارات البيولوجية على المركبات الجديدة ووجد أن لها تأثير مضاد لل dna وكذلك بالمقارنة بالمضادات (دوكسوروبسين) كمراجع وجدت نتائج هذه المركبات على النحو التالي:

**وقد جاءت نتائج هذه المركبات على النحو التالي:**

بالنسبة لنشاط المضادة للسرطان فقد كانت أقوى النتائج هي للمركب (4) حيث أظهر فاعلية كبيرة ضد جميع الخلايا

وقد أظهرت نتائج تقييم السمية لهذه المركبات على النمو التالي، بالنسبة لنشاط المضادة للسرطان فقد كانت أقوى النتائج هو المركب 9 حيث أظهر التأثير الأكبر ضد جميع الخلايا السرطانية المختبرية بنتائج تتراوح بين 12.09 الي 27.83 ميكرومول وبالمقارنة بقيم نتائج الدوكسوروبسين كعقار ممّكح التي تتراوح بين 4.57 إلي 0.57 ميكرومول بينما أظهر المركبات 10 و13 فيما متوسطه تراوح بين 12.06 إلي 38.27 ميكرومول و 57.21 الي 98.81 ميكرومول بالترتيب بالمقارنة بالدوكسوروبسين أما بأيا المركبات فكانت نتائجها أقل قوًة مقارنة بالمركبات الأخرى.

**الكلمات المفتاحية:** مثبطات مستقبل عامل نمو البشرة، مشتقات البيرازولوبريميدين، مضادات للسرطان