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

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ABSTRACT

A series of novel pyrazolo[3,4-*d*]pyrimidine derivatives have 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 IC_{50} values ranging from (4.03- 6.18) µM comparable to that of doxorubicin as a control drug (IC_{50} values of 8.17 and 9.27 µM). In particular, compounds (**8**,**9**,**11) and (12)** exhibited higher intercalative activity with IC_{50} value of 30 µM than doxorubicin (31 µM).

Keywords: Anticancer, pyrazolopyramidine 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).

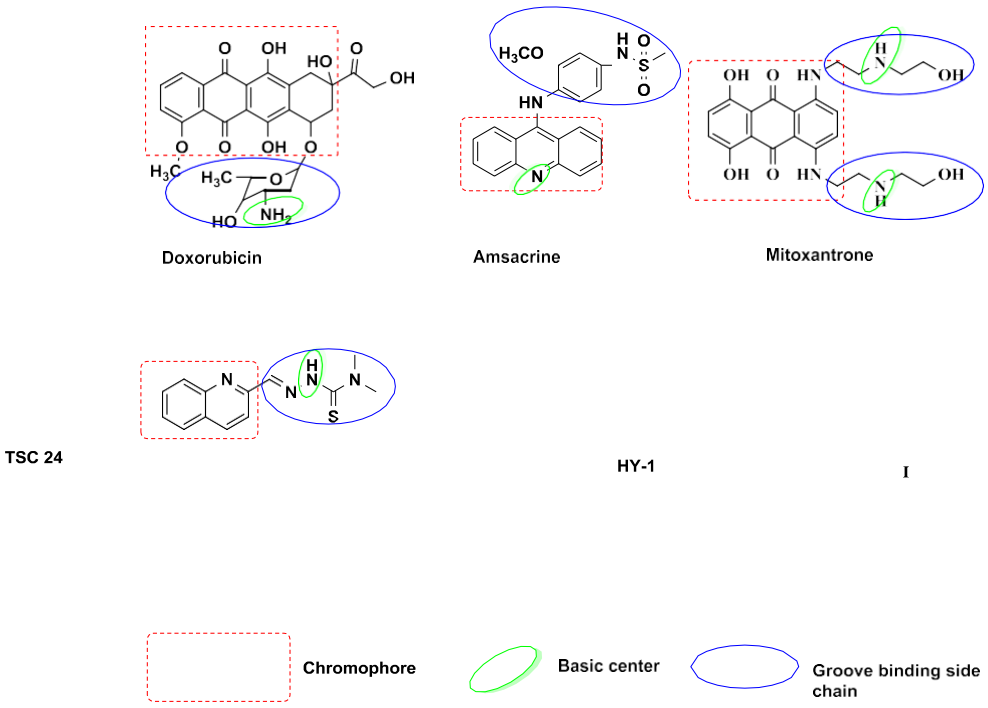
These class of drugs act either by topo poisoning via inter-chelation with DNA as doxorubicin (Liu *et al*. 1989), a msacrine (Sung *et al*. 2005) and mitoxantrone (Shenkenberg *et al*. 1986). On the other hand, drugs act as catalytic inhibitors of Top-II as TSC24 (Huang *et al*. 2010), HY-1 (zhao *et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

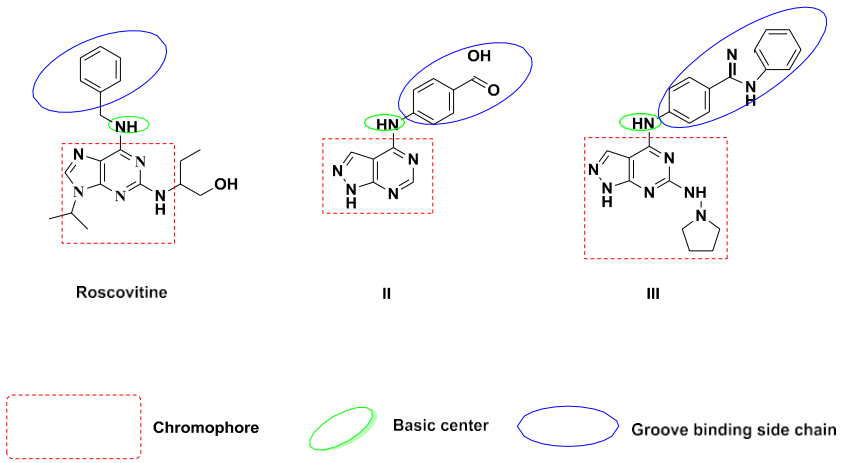
cationic center may be an amino or nitrogen containing heterocyclic group, which can be protonated at physiological pH (Lee *et al*. 2017). The third feature is a flexible side chain that anchors DNA (Bailly *et al*. 2012) (Figure1).

On the other hand, pyrazolopyramidine 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). Pyrazolopyramidine 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 IC_{50} value of 1.30 µM. besides, its topoisomerase inhibition (Pogorelčnik *et al*. 2015) (Figure2).



(Figure 1). DNA intercalators and their basic pharmacophoric features



(Figure 2). Pyrimidine derivatives as topoisomerase II Inhibitors

Compound (**III)** proved to be the most active and efficacious candidate in this series, with mean IC_{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_{50} value of 2.7 μM (Whittaker *et al*.2004).

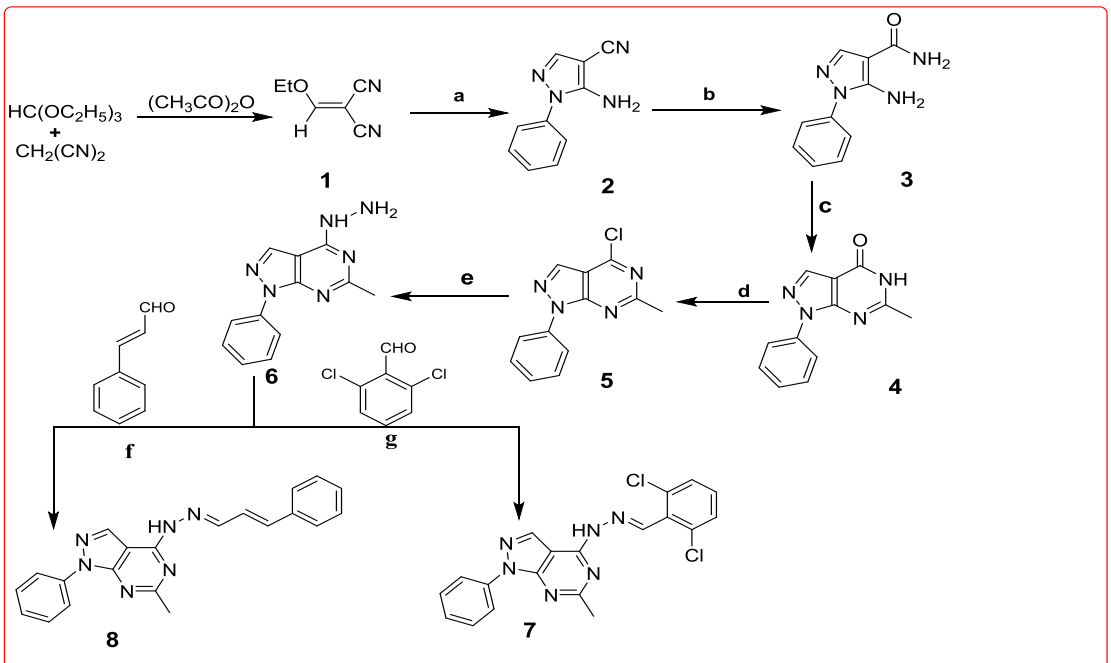
Therefore, on the basis of previously above-mentioned findings and in resumption of our previous efforts in the design and synthesis of new anticancer agents (Gaber *et al*. 2018), we report the design, synthesis and anticancer activities of new pyrazolo[3,4-*d*]pyrimidine derivatives. These derivatives 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-1*H*-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-4*H*-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-4*H*-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 ^{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-dichlorobenzaldahyde and cinnamaldehyde in absolute ethanol with catalytic amount of glacial acetic acid to give the corresponding hydrazones (**7**) and (**8**) respectively.

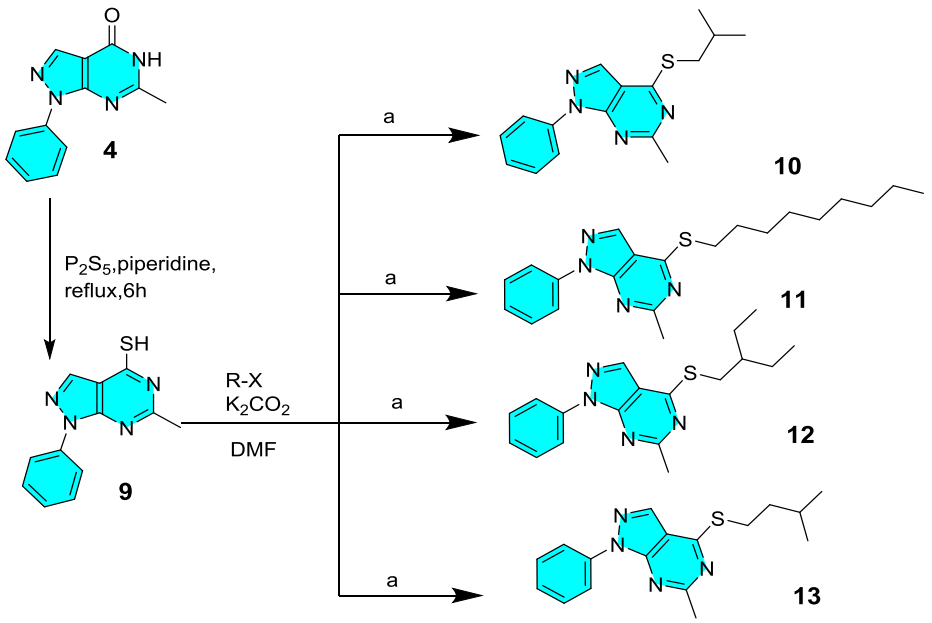


(Scheme 1).

General procedure for synthesis of pyrazolo[3,4-d]pyrimidine derivatives (1-8). Reagents and conditions of reaction; (a) phenyl hydrazine, absolute ethanol, reflux, 2 h; (b) sodium hydroxide, absolute ethanol, HCl, reflux, 5 h; (c) ethylacetate, sodium ethoxide, absolute ethanol, HCl, reflux, 14 h; (d) phosphoryl trichloride, reflux, 6 h; (e) hydrazine hydrate 99%, reflux, 8 h; (f) cinnamaldehyde (0.001 mol) and catalytic amount of glacial acetic acid (0.5 ml) and (g)2,6-dichlorobenzaldahyde and catalytic amount of glacial acetic acid (0.5 ml)

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, namely1-bromoisobutan, 1-bromononane, 1-bromo-3-methylpentane, 1-bromo-2-ethylebutane 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)



Scheme 2. Reaction of the thiole derivative 9 with alkyl halides
Reagents and conditions of reaction; (a) appropriate alkyl bromide, anhydrous potassium carbonate and DMF

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}). H^1 NMR and C^{13} NMR spectra were performed on Gemini 300BB spectrometer at 300MHz and Bruker spectrometer at 75MHz, respectively. TMS was used as internal standard and DMSO*d*6 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).

Amixture of hydrazine derivative (**6)** (0.30 g, 0.001 mol), 2,6-dichlorobenzaldahyde, 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-1*H*-pyrazolo[3,4-*d*]pyrimidine (7)

Red solid; reaction time: 6 h; yield 83% (0.38 g); m.p.: 255-257°C; IR (KBr, *v*, cm^{-1}), 3383(NH), 3109 (Ar-H), 2910(aliph-CH).^{1}HNMR(DMSO-d_6,): 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- CH_3).^{13}C NMR (DMSO, d_6): 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^{+4}, 76.45%), 396.33 (M^{+2}, 100%); Anal. Calcd.for $C_{19}H_{14}Cl_2N_6$ **(**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-((1*E*,2*E*)-3-phenylallidene) hydrazinyl)-1*H*-pyrazolo[3,4-*d*] pyrimidine (8)

Orange needle, reaction time: 5 h; yield 95% (0.39 g); m.p.: 241- 243°C; IR 7(KBr, *v*, cm^{-1}). 3317(NH), 3112 (Ar-H), 2886 (aliph-CH).^{1}H NMR (DMSO-d_6,): 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=C**H**-ph), 6.89 (d, 1H, *J* = 7.5 Hz, N=CH-C**H**=CH), 2.35 (s, 3H, pyrimidine-CH_3). ^{13}C NMR (DMSO-d_6,): 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 $C_{21}H_{18}N_6$ **(**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-1*H*-pyrazolo[3,4-*d*]pyrimidine-4-thiol (9)

Phosphorus pentasulfide (1.67 g, 3.75 mmol) was suspended in 40 ml of pyridine and compound **(4)** (0.5 g, 3.1 mmol) was added. The reaction mixture was refluxed for 5h. then 200 ml of worm water was added to the contents of this flask. The precipitate 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, *v*, cm^{-1}): 3009 (C-H aromatic), 2933 (C-H aliphatic), 1498 (C=N). ^{1}HNMR (DMSO-d_6), 13.15 (s, 1H, SH D_2O 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-CH_3).^{13}C- NMR (DMSO-d_6): 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 $C_{12}H_{10}N_4S$ **(**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-1*H*-pyrazolo[3,4-*d*]pyrimidine (10)

Yellwish crystals, (2.2 g; 76%); m.p. 80-83^{0}C; IR (KBr)cm^{-1} : 3017 (C-H aromatic), 2957 (C-H aliphatic), 1563 (C=N) .^{1}H-NMR (DMSO-d_6) 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-**CH$_2$**), 2.51 (s, 3H, pyrimidine-CH$_3$), 1.05 (m, 1H, **CH**-(CH$_3$)$_2$), 0.91 (d, 6H, *J* = 6.8 Hz, CH-((**CH$_3$**)$_2$).^{13}C NMR (DMSO-d_6): 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 ($C_{16}H_{18}N_4S$): C, 64.40; H, 6.08; N, 18.78; Found: C, 64.45; H, 6.12; N, 18.90.

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

Yellow crystals, (2.20 g; 70%); m.p. 85-95^{0}C; IR (KBr)cm^{-1}: 3072 (C-H aromatic), 2925 (C-H aliphatic), 1559 (C=N). ^{1}H-NMR (DMSO-d_6) 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- CH$_3$), 125-1.77(m, 6H, (CH$_2$)$_3$), 1.23-1.45 (m, 6H-(CH$_2$)$_3$), 0.86 (t, 3H, *J* = 7.2 Hz).^{13}C NMR (DMSO-d_6): 165.1, 164.8, 152.1, 152.1, 138.8, 133.7, 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 ($C_{21}H_{28}N_4S$): C, 68.44; H, 7.66; N, 15.20; Found:C,68.41; H, 7.69; N, 15.24.

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

Brownish crystals,(2.20 g; 72%); m.p. 85-90^{0}C; IR (KBr)cm^{-1}: 3072 (C-H aromatic), 2962 (C-H aliphatic),1560 (C=N).^{1}H-NMR (DMSO-d_6): 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-CH$_2$), 2.51 (s, 3H, pyrimidine-CH$_3$),1.60 (m,5H, 2**CH$_2$**), 0.93 (t, 6H, *J* =7.5 Hz , 2CH$_3$).^{13}C NMR (DMSO-d_6): 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 ($C_{18}H_{22}N_4S$): 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-1*H*-pyrazolo[3,4-*d*]pyrimidine (13)

brownish crystals, (2.23 g; 75%); m.p. 80-87^{0}C; IR (KBr)cm^{-1} : 3076 (C-H aromatic), 2955 (C-H aliphatic),1550 (C=N).^{1}H-NMR (DMSO-d_6) 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-**CH$_2$**), 2.51 (s, 3H, pyrimidine-CH$_3$), 1.74 (m, 2H, **CH$_2$**-CH-((CH$_3$)$_2$), 1.65 (m, 1H, **CH**-(CH$_3$)$_2$, 0.95 (d, 6H, *J* = 6.8 Hz, CH-(**CH$_3$**)$_2$).^{13}C NMR (DMSO-d_6); 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 ($C_{17}H_{20}N_4S$): 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, 4.17and 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)**: 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak)

and above 100 (non-cytotoxic)

••• **DOX** Doxoru:bicin

for IC50 values of the active compounds are summarized in Table 1.

Cytotoxic activity of some compounds against human tumor cells

	In vitro Cytotoxicity 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>**

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 IC_{50} values of 4.50, 4.17 and 5.13 μM against tested cellines 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 البيرازولو(٣,٤-د) بيريميدين الجديدة المتوقع لها نشاط مضاد للسرطان

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في هذا البحث تم تصميم وتحضير بعض مشتقات البيبرازولو (٣,٤-د) بيبراميدين الجديدة من خلال بعض المركبات الوسيطة وقد تم تقيم النشاط المضاد للسرطان لهده المركبات الجديدة في مقابل بعض انواع السرطان كسرطان الثدي وسرطان الكبد وسرطان القولون وكدلك تم تقيم كفاءة هده المركبات كمتدخلات مع الحمض النووي وقد تم اختيار انشط هده المشتقات لاختباره كمثبط لانزيم توبوايزوميرازاثنان.

وتم إثبات الصيغ البنائية للمركبات باستعمال جهاز الآشعة دون الحمراء وجهاز الرنين النووي المغناطيسي ومطياف الكتلة هذا بالإضافة إلى التحليل الدقيق لعناصر المركبات التى تبين نسبة الكربون والهيدروجين والنتروجين فى المركبات وتم إجراء الاختبارات البيولوجية على المركبات الجديدة فوجد أن لها تأثير مضاد للورم وذلك بالمقارنة بالمضاد للسرطان (دوكسوروبيسين) كمرجع

وقد جاءت نتائج هذه المركبات على النحو التالي:

بالنسبة للنشاط المضاد للسرطان فقد كانت اقوى النتائج هي للمركب (٩) حيث اظهر فاعلية كبيرة ضد جميع الخلايا

وقد اظهرت نتائج تقييم السمية لهذه المركبات على النحو التالي، بالنسبة للنشاط المضاد للسرطان فقد كانت اقوى النتائج هي للمركب ٩ حيث اظهر التأثير الابرز ضد جميع الخلايا السرطانيةالمختبرة بنتائج تتراوح بين ١٢.٠٩ الى ٢٧.٨٣ ميكرومول وبالمقارنة بقيم نتائج الدوكسوروبيسين كعقار محكم التي تتراوح بين ٤.٥ الى ٥.٥٧ ميكرومول بينما اظفر المركبات ١٠ و١٣ قيما متوسطه تتراوح بين ١٢.٠٩الى ٣٨.٠٦ ميكرومول و ٧٥.٢٦ الي ٩٨.٨١ ميكرومول بالترتيب بالمقارنة بالدوكسوروبيسين اما باقي المركبات فكانت نتائجها اقل قوه مقارنة بالمركبات الاخرى.

الكلمات المفتاحية: مثبطات مستقبل عامل نمو البشرة، مشتقات البيرازولوبيريميدين ، مضاد للسرطان