IJPSR (2017), Vol. 8, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 August, 2016; received in revised form, 03 October, 2016; accepted, 06 December, 2016; published 01 February, 2017

SYNTHESIS AND ANTICANCER SCREENING OF HETEROCYCLIC COMPOUNDS BEARING PYRIMIDO [4, 5-B] QUINOLINE MOIETY

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Keywords:

2-aminoquinoline-3-carbonitrile, Pyrimido [4, 5-b] quinolines, *in vitro* antitumor screening, docking

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ABSTRACT: Due to your efforts diligent on quinoline nucleus new series containing pyrimido [4, 5-b] quinoline were synthesized via starting with the strategic 2-aminoquinoline-3-carbonitrile (2) intermediates to afford various pyrimido [4, 5-b] quinoline via cyclization with different reagents to get compounds 3-24. Compounds 3, 6, 9a, 9b, 12, 15, 16a, 16b, 17, 21 and 24 were tested for *in vitro* antitumor activity against human breast carcinoma (MCF-7) cell line, We found what we were hoping for where compound 6 was found the most active member (IC50= 48.54 μM) than the Doxorubicin as a reference drug with (IC50= 71.80 μM). To understand the interaction of binding sites with the target protein receptor, the docking study was performed using topoisomerase II. The results of in vitro cancer activity and docking study revealed that the synthesized compounds have potential cancer activity and can be further optimized and developed as a lead compound.

INTRODUCTION: Quinoline skeleton is often used for the design of many synthetic compounds with diverse pharmacological properties. Quinoline and its derivatives are receiving important due to their wide range of biological activities as a drug analgesics [1], antitubercular [2], antiallergics [3], anticonvulsant [4], antihistaminic [5], antibacterial [6], in addition, other derivatives also exhibit good antitumor activity [7-10]. In the light of these valid observations, pyrimido quinoline derivatives continue to attract the interest of medicinal chemists due to the wide range of biological properties. Because some of them showed antitumor activity [11-16]. The recognition of antibiotics as an important class of antitumor agents is quite recent [17].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(2).570-81

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (2).570-81

The antitumor antibiotics owe their cytotoxic action primarily to their interactions with DNA, leading to disruption of DNA function. In addition to intercalation, their abilities to inhibit eukaryotic topoisomerase II [18]. Drugs targeting has been termed topoisomerase II poisons which includes anthracyclines (e.g. Doxorubicin and Mitoxantrone) [19, 20]. These agents generate a "lesion" that includes DNA strand breaks and protein covalently bound to DNA[21]. Herein, we report synthesis of certain derivatives of pyrimido [4, 5-b] quinoline that have the basic pharmacophoric feature of topoisomerase II binding agents and antineoplastic evaluation against human breast carcinoma (MCF-7) cell line.

Rationale of molecular design: We depend on ligand based drug design particularly a molecular hybridization approach that involves the coupling of two or more groups with relevant biological properties [22] quinoline nucleus was hybridized with pyrimidine moiety to afford pyrimido [4, 5-b] quinoline scaffold, which was subjected for several chemical modification via chemical substitution on

pyrimidine ring to produce many derivatives with different chemical isosters in order to study structure relationships (**Fig. 1**). In addition, we hope to get new, potent, safe and effective anticancer agents with topoisomerase II binding ability. Of note, many studies reported useful data about the binding pattern of doxorubicin with the active site of top-2. [23, 24] Utilizing these data we will carry out molecular docking to justify the inhibitory action of our target compounds towards topoisomerase II.

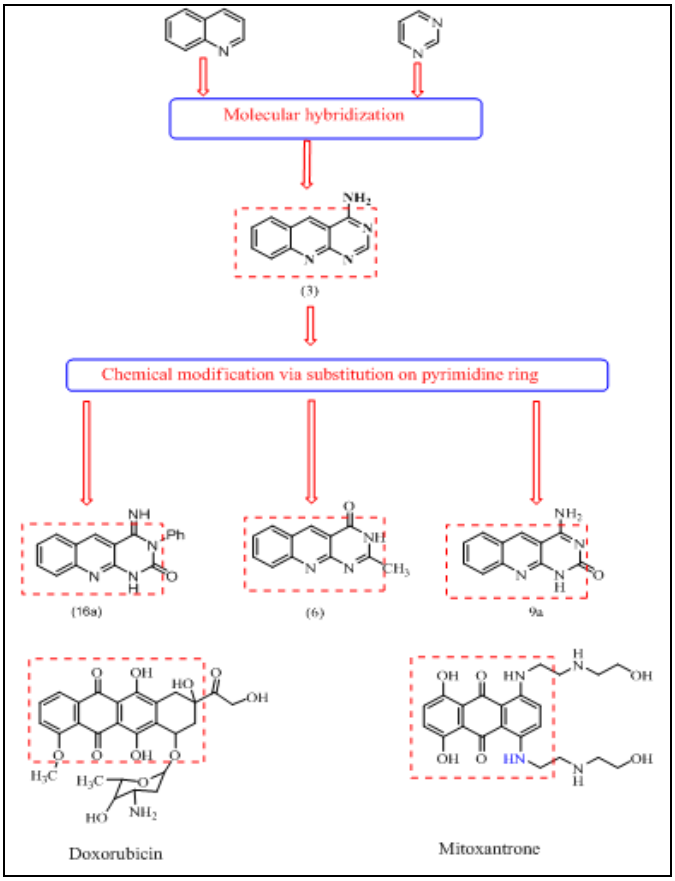


FIG. 1: RATIONALE OF DESIGN OF NEW ANTICANCER AGENTS AND CHEMICAL MODIFICATIONS

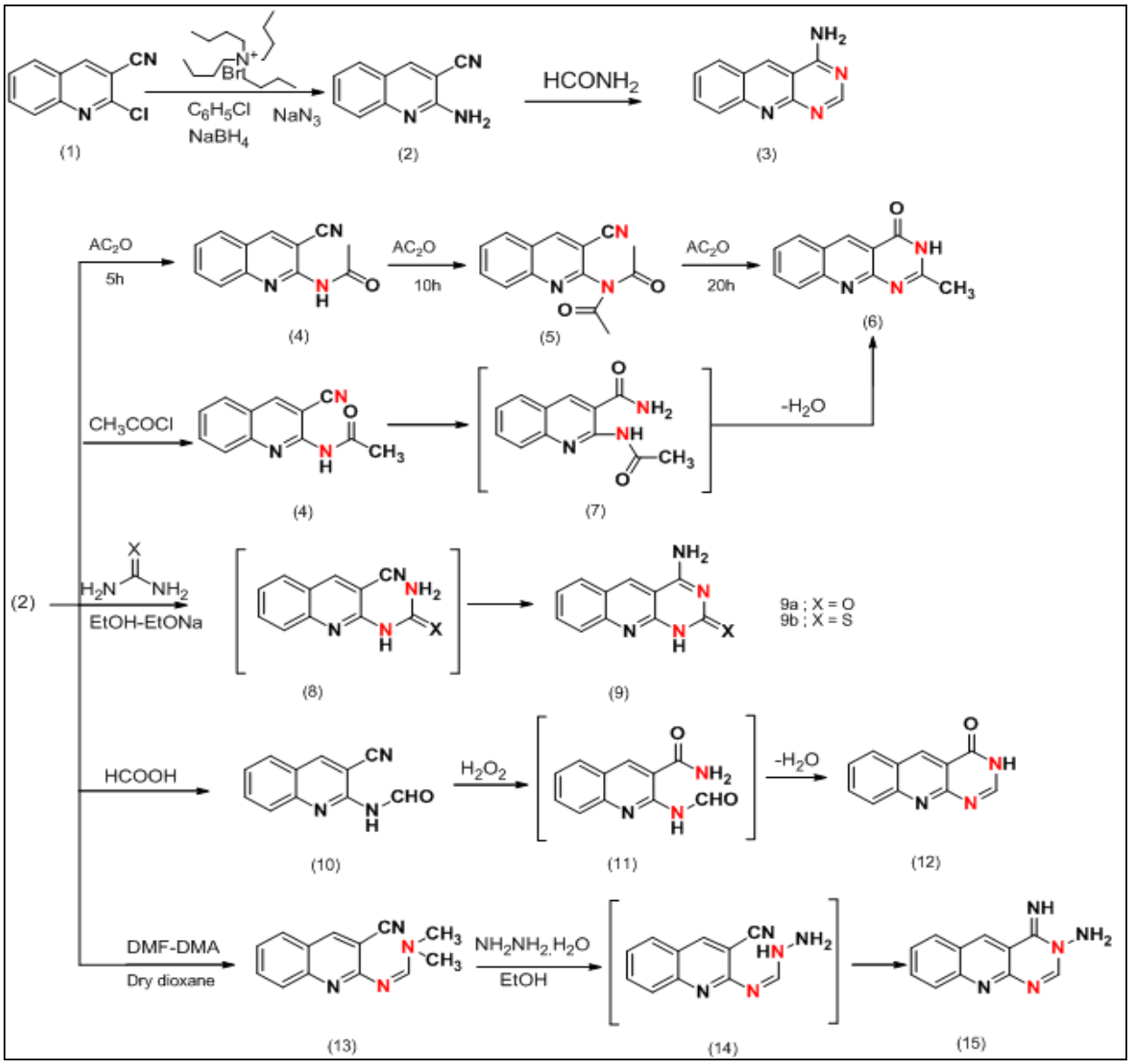
RESULTS AND DISCUSSION: The synthetic method adopted to obtain the reported compound 2 that was prepared by nucleophilic substitution reaction [23] and that was reacted with formamide to afford the pyrimido[4,5-b]quinoline derivatives 3 where cyclization occurred through elimination of one molecule of water, followed by intramolecular cyclization. The structure of compounds 3 were established on the basis of elemental analysis and spectral data, where IR spectra of these compound revealed the absence of (C≡N) bands, which confirms the cyclization and the formation of the pyrimido[4,5-b]quinoline systems.

When key compound 2 were refluxed with acetic anhydride, different products were obtained according to the time of the reaction but we focused here on the product containing pyrimido [4,5-b]quinoline moiety 6 that obtained by refluxing in acetic anhydride for 15 hours or acetyl chloride followed by neutralization with acid to give 6. The structure of compound 6 was confirmed by the absence of the cyano group and the presence of bands at 1703 cm^{-1} corresponding to carbonyl group. Furthermore, ^{1}HNMR spectrum of compound 6 revealed the methyl group protons as one singlet at 2.33 ppm. When compound 2 reacted with thiourea or urea it afford pyrimido [4, 5-b] quinoline–2(1H)– one/thione derivatives 9a and 9b (**Scheme 1**). The reaction products 9 were formed via the intermediate 8 by loss of an ammonia molecule, followed by an intramolecular addition to the cyano function to give the final isolated products 9a, b. IR spectra confirm the structures of 9a and 9b which indicated the disappearance of the cyano group, and the appearance of (C=O) at 1616 cm^{-1} for 9a and (C=S) at 1263 cm^{-1} for 9b. Another pyrimido [4, 5 - b] quinoline derivative 12 were obtained by refluxing compound 2 in formic acid to afford compound 10 that cyclized into 12 via condensation with alkaline hydrogen peroxide to give the corresponding intermediate 11.

The reaction proceeds by initial hydration of the nitrile group to give a carboxamide, followed by elimination of two moles of water .IR spectrum of compound 12 revealed the absence of the band corresponding to the cyano group and the presence of band at 1701 cm^{-1} corresponding to C=O group . Additionally, ^{1}HNMR spectrum of 12 showed the presence of singlet at 7.9 ppm for NH, and singlet at 8.09 ppm for CH=N, and the absence of the signal corresponding to the amino group. Furthermore, pyrimido [4,5-b]quinoline derivative 15 were obtained by reaction of 2 with dimethyl amine to produce 13 that when reacted with hydrazine hydrate it was cyclized into 15 via intermediate 14.

The IR spectrum of compound 15 showed two absorption bands at 3421, 3148, for (NH_2) and (NH) groups, respectively. Also, it showed the disappearance of the (C≡N) absorption band. The ^{1}HNMR spectrum of the same compound revealed the presence of three singlets at d 5.40, 7.65, and

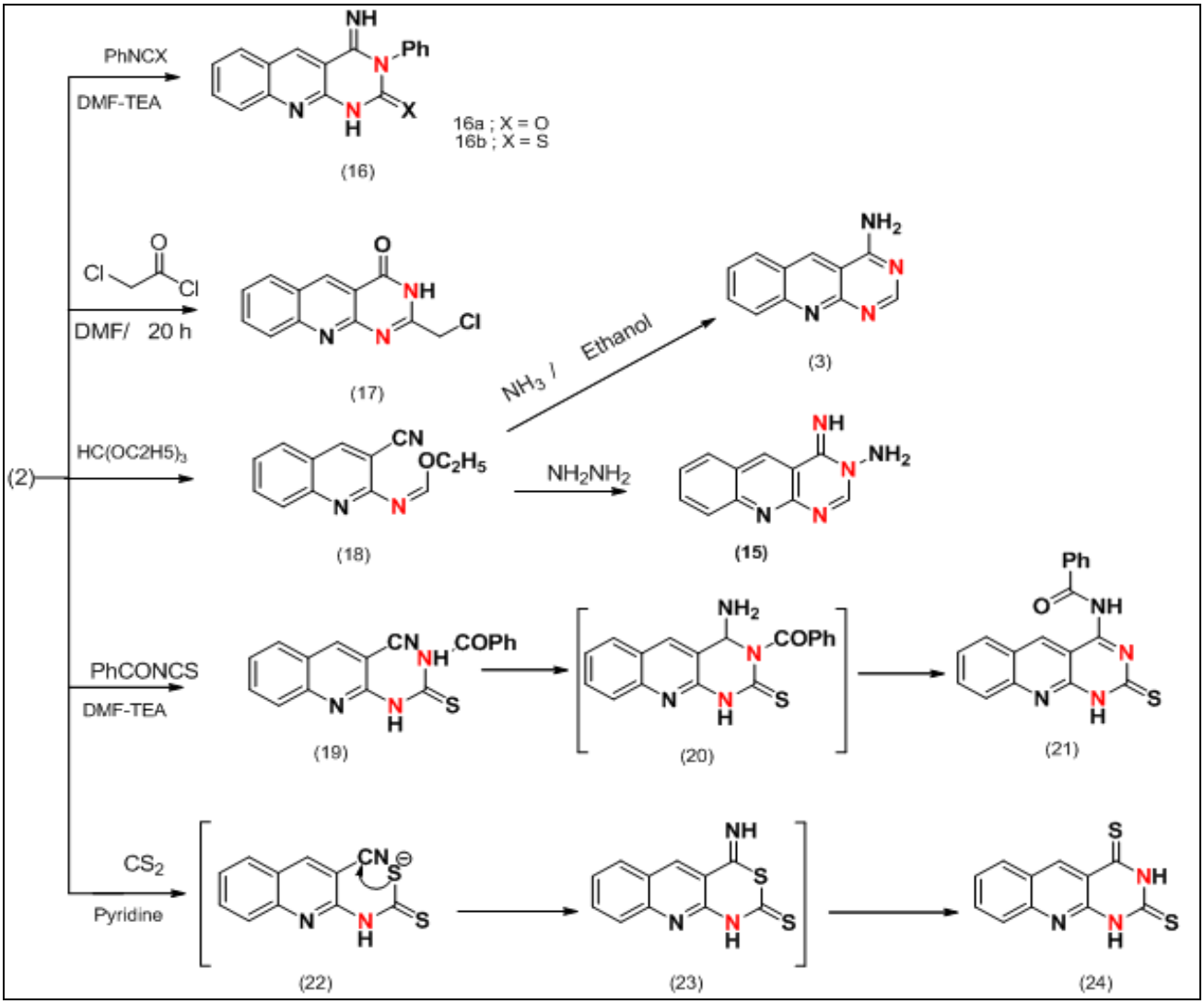
8.2 ppm indicating (NH_2), (C5–H) and (NH) groups, respectively. In addition, the remaining aromatic protons appeared at the expected regions. In addition, compound 2 was refluxed with isocyanates and isothiocyanates to give initially the corresponding substituted ureido or thioureido derivatives which readily cyclized to give the corresponding fused heterocyclic systems 16a, b (**Scheme 2**). The IR spectrum of 16a, b showed the absence of (C≡N) band and displayed two absorption band at the range 3099-3170 cm^{-1} due to (2NH) that confirming the formation of the desired cyclic structures. Moreover, the signals indicating (C5–H) and aromatic



SCHEME 1: SYNTHESIS OF COMPOUNDS 1-15

Protons were observed at the expected regions. Refluxing compound 2 with chloroacetyl chloride in dimethyl formamide it afford 2-(chloromethyl) pyrimido[4,5-b]quinoline derivative 17.Also, IR spectrum of compound 17 revealed the absence of the band corresponding to cyano group, and the presence of band corresponding to one carbonyl groups at 1670 cm^{-1}. Furthermore, ^{1}HNMR spectrum of compound 17 revealed the 2-chloromethyl group protons as one singlet at 4.9 ppm, corresponding to two protons, and a singlet for NH at 8.10 ppm. It must be indicated that compound 18 were obtained from reaction of 2 with triethyl orthoformate which can be cyclized into compound 3 when it react with ammonia and can be cyclized to give the compound 15 when compound 18 react with hydrazine.

A new pyramid [4,5-b]quinoline were obtained when compound 2 reacted with benzoylisothiocyanate to yield 19 which then cyclized into 20 intermediate which undergoes a Dimroth rearrangement to give adesired 21. The IR spectrum of 21 showed the disappearance of (C≡N) band and appearance of two absorption band at 3180 and 3117 due to (2NH) in addition, to two band one at 1668 cm^{-1} due to (C=O) and another one at 1360 cm^{-1} due to (C=S). Furthermore, the mass spectrum of compound 21 showed peaks at m/z 338 that confirming the formation of the desired cyclic structures 21. Finally, 2 were further utilized for another cyclocondensation reaction using carbon disulfide in pyridine to afford the pyrimido [4, 5-b] quinoline-2,4(1H, 3H)-dithiones 24 (**Scheme 2**). The structural assignments of compounds 24 were based on microanalytical and spectral data. The IR spectra showed the disappearance of the (C≡N) absorption band and the mass spectrum showed peaks at m/z 245 confirming the formation of the desired cyclic structures. Regarding the ^{1}HNMR spectrum of the same compound, two singlets at 5.75 and 9.62 ppm that exchangeable with D_2O indicating (2NH) were clearly identified in addition, to one singlet at 8.86 due to C5-H. Also aromatic protons appeared at the expected regions.



SCHEME 2: SYNTHESIS OF COMPOUNDS 16-24

Biological Evaluation: Cell line (MCF-7) Cells were placed in 96-multiwell microliter plate (104 cells/ well) for 24 h before treatment with the compounds to allow attachment of the cells to the wall of the plate. Different concentrations of the tested compounds (0, 10, 25, 50 and 100 µM) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 0C and in atmosphere of 5 % CO_2.

After 48 h, cells were fixed, washed and stained with SRB stain. Unbound dye was removed by four washes with 1 % acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader at a wave length of 570 nm. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time, using Microsoft Office Excel 2003. The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared to the reference drug doxorubicin (CAS, 25316-40-9)

Molecular Modeling: Docking was carried out on topoisomerase II protein downloaded from protein data bank (PDB), (code 1zxm, resolution 1.87 Å), using discovery studio 2.5 software. The 3D crystal structure of topoisomerase receptor (code 4BUL) was downloaded from PDB, water molecules were removed. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Protein was subjected to energy minimization and applying of CHARMM (Chemistry at HARvard Macromolecular Mechanics) force fields for charge, and MMFF94 (Merck Molecular force field) force field for partial charge. Inflexibility of structure is obtained by creating fixed atom constraint. The binding site of the protein was defined and prepared for docking. Doxorubcin, a reference drug, and the designed compounds 2D structures were sketched using ChemBioDraw Ultra 14.0 and saved in MDL-SDfile format. SDfile opend, 3D structures are protonated and energy minimized by applying CHARMM force fields for charge, and MMFF94 force field for partial charge, then prepared for docking by optimization the parameters. Molecular docking was performed using (CDOCKER) protocol which is an implementation of the CDOCKER algorithm. CDOCKER is a grid-based molecular docking method that employs CHARMM-based molecular dynamics (MD) scheme to dock ligands into a receptor binding site. The receptor is held rigid while the ligands are allowed to flex during the refinement.

Antitumer Screening: Cell line (MCF-7) Cells were placed in 96-multiwell microliter plate (10^4cells/well) for 24 h before treatment with the compounds to allow attachment of the cells to the wall of the plate. Different concentrations of the tested compounds (0, 10, 25, 50 and 100 µM) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 ^{0}C and in atmosphere of 5 % CO_2. After 48 h, cells were fixed, washed and stained with SRB stain. Unbound dye was removed by four washes with 1 % acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader at a wave length of 570 nm. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time, using Microsoft Office Excel 2003. The molar concentration required for 50% inhibition of cell viability (IC50) was calculated and compared to the reference drug doxorubicin (CAS, 25316-40-9). The surviving fractions were expressed as means ± standard error and the results are given in **Table 1.**

TABLE 1: *IN VITRO* ANTITUMOR SCREENING OF THE SELECTED COMPOUNDS AGAINST HUMAN BREAST CANCER CELL LINE (MCF-7)

Compound	Compound Concentration (µM)				IC50 (µM)
	10	25	50	100	
	Surviving Fraction (Means ± SE)#				
Doxorubicin	0.721±0.02	0.546±0.02	0.461±0.01	0.494±0.03	71.80
3	0.811±0.03	0.521±0.05	0.352±0.01	0.402±0.01	58.47
6	0.712±0.03	0.533±0.01	0.391±0.02	0.245±0.03	48.54
9a	0.786±0.02	0.534±0.04	0.472±0.04	0.343±0.02	58.77
9b	0.772±0.03	0.515±0.02	0.473±0.04	0.355±0.01	58.73
12	0.755±0.02	0.601±0.03	0.506±0.05	0.431±0.04	70.33
15	0.872±0.03	0.636±0.04	0.491±0.03	0.332±0.04	63.01
16a	0.866±0.03	0.643±0.03	0.442±0.03	0.379±0.02	69.24
16b	0.911±0.02	0.654±0.03	0.511±0.03	0.430±0.04	72.87
17	0.955±0.04	0.801±0.04	0.606±0.03	0.431±0.02	80.55
21	0.961±0.04	0.801±0.05	0.606±0.02	0.561±0.04	99.10
24	0.968±0.05	0.891±0.03	0.661±0.05	0.493±0.01	92.74

#: Each value is the mean of three values ± Standard Error.

Biological Activity: The *in vitro* anticancer screening against breast cancer (MCF-7) cell line was done at the National Cancer Institute (NCI), Cancer Biology Department, Pharmacology Unit, Cairo, Egypt, adopting the sulforhodamine B (SRB) assay [26] using doxorubicin as a reference antitumor agent. The results of *in vitro* antitumor activity of the tested compounds indicated that compound 6, 3, 9b, 9a, 15, 12 and 16a exhibited the highest cytotoxic activity against human breast cancer cells (MCF-7) with IC50 value 48.54, 58.47, 58, 73, 58.77, 63.01, 70.33 and 69.24 respectively. Compounds 16b, and 17 showed lowest activity with IC50 values of 72.87and 80.55 respectively. On the other hand, compounds 24 and 21 exhibits lowest cytotoxic activity when it compared with the reference drug doxorubicin (IC50= 71.8 µM).

Discussion of Modeling: The obtained results indicated that all studied ligands have similar position and orientation inside the putative binding site of type topoisomerase II protein. The selected compounds (3, 6, 9a, 9b and 21) showed good binding energies ranging from – 39.80to -45.45 kcal/mol (**Table 2**). The proposed binding mode of the ligand (doxorubcin) binding free energy was -55.88 kcal/mol with RMSD value of 2.5. It showed the important interactions with the residues at the active site of type topoisomeraseII protein (figure **2**A). It formed nine hydrogen bonds of with Ser148, Ser149, Asn91, Ala187, and Asn120 aminoacid. The proposed binding mode of compound **3** (affinity value of – 41.38 kcal/mol and 1 H-bond) is virtually the same as that of *doxorubicin* (**Fig. 2B**). It formed a hydrogen bond with Asn91. The proposed binding mode of compound **6** (affinity value of – 45.45 kcal/mol and three H-bonds) is virtually the same as that of *doxorubcin* (**Fig**. **2C**). It formed a hydrogen bond with Ser148, Ser149, and Asn150.

The proposed binding mode of compound **9a** (affinity value of – 40.44 kcal/mol and two H-bonds) is virtually the same as that of *doxorubcin* (**Fig. 2D**). While compound **9b** formed three H-bonds(affinity value of – 40.22 kcal/mol) (**Fig**. **2E**) Finally, The proposed binding mode of compound **21** (affinity value of – 39.80 kcal/mol and four H-bonds) is virtually the same as that of *doxorubcin* (**Fig. 2F**). These interactions of compounds **3, 6, 9_a,** and **9_b** with topoisomerase II protein explain the high binding free energies and the biological activities.

TABLE 2: THE DOCKING BINDING FREE ENERGIES OF THE SYNTHESIZED COMPOUNDS

Comp. No.	Binding free energy (kcal/mol)	Comp. No	Binding free energy (kcal/mol)
3	- 41.38	19	-35.60
4	-18.10	21	-39.80
5	-31.45	24	– 36.63
6	-45.45	15	-29.10
9_a	-40.44	16_a	-24.50
9_b	-40.22	16_b	-20.90
10	-32.50	17	– 33.11
12	-33.23	18	-29.36
13	-32.70	*Doxorubcin*	-55.88

Structure activity relationship: Observing the results, we could deduce valuable data about the structure activity relationships. Firstly, we explored the effect of the substitution on 4- position of Pyrimido [4, 5-b] quinoline moiety. The decreased IC_{50} value of compound 3, with incorporated amino group, (58.47µM) than the corresponding member as compound 12 with carbonyl group (70.33 µM) and compound 24 with thione group (92.74 µM) indicated that substitution with amino group is more advantageous than carbonyl and thione groups. Moreover, introduction of benzoyl moiety (more bulky than hydrogen) at this amino group resulted in remarkable decrease of activity for compounds 21 (IC_{50} = 99.10 µM).We then investigated the impact of the substitution on 2-position of pyrimido [4, 5-b] quinoline. It was found that substitution with methyl, carbonyl, or thione moieties as compounds 6, 9a, and 9b with IC_{50} (48.54, 58.77, and 58.73), respectively, increases the anticancer activity when compared with that of unsubstituted one. Finally, substitution at 3- position of pyrimido [4, 5-b] quinoline nucleus with amino or phenyl moieties decreases the anticancer activity.

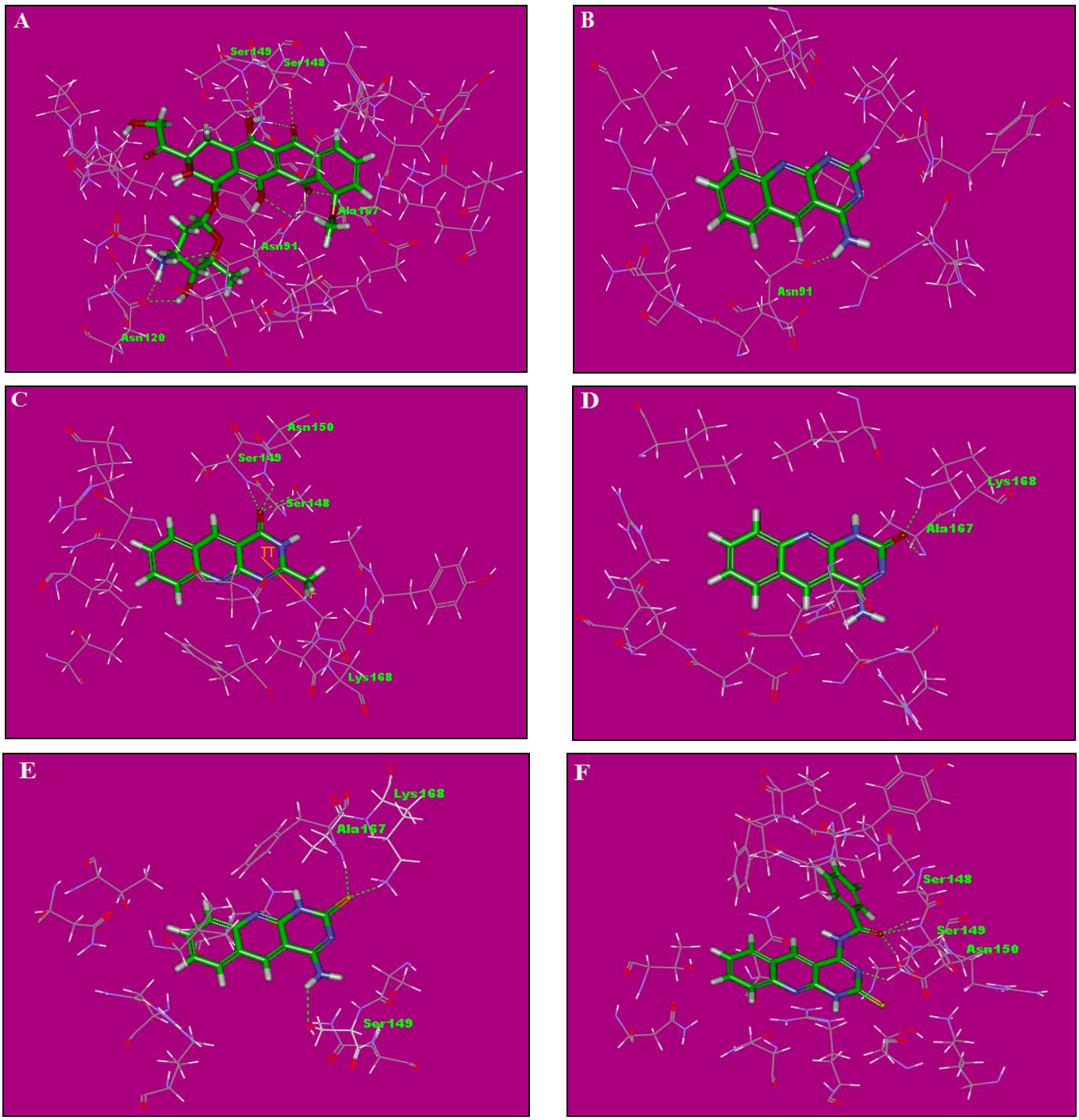


FIG. 2: A) BINDING INTERACTION OF DOXORUBCIN. B) BINDING INTERACTION OF COMPOUND 3. C) BINDING INTERACTION OF COMPOUND 6. D) BINDING INTERACTION OF COMPOUND 9a E) BINDING INTERACTION OF COMPOUND 9b. F) BINDING INTERACTION OF COMPOUND 21

CONCULOSION From the result in the **Table 1** new series of pyrimido [4, 5-b] quinolines have been synthesized and evaluated for in vitro antitumor activity against human breast carcinoma (MCF-7) cell line. The results of in vitro antitumor testing against MCF-7 breast cancer cells indicated that compounds 6, 3, 9b, 9a, 15, 12, and 16a exhibited better activities among screened compounds.

General Procedure: Melting points were measured in capillary tube on a Graffin melting point apparatus and are uncorrected. The IR spectra were recorded on Pye Unicam SP 1000 IR spectrophotometer using KBr discs (λmax in cm-1). ^{1}HNMR spectra were performed on Gemini 300BB (300MHz) and 300 MHz for ^{13}C NMR), spectrometer, using TMS as internal standard and DMSO-d6 as solvent; the chemical shifts are

reported in ppm (δ) and coupling constant (J) values are given in Hertz (Hz). Signal multiplicities are 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 Hewlett Packard 5988 spectrometer at the RCMB. The purity of the compounds was checked by thin layer chromatography (TLC) on Merck silica gel 60 F254 precoated sheets.

All analyses were performed at the Micro-analytical Unit of Cairo University, Cairo, Egypt. Compounds 1 were prepared according to the reported procedure [19-22] and compound 2 were prepared adopting the described procedures [23]. The following materials were used in the biological screening: Breast cancer (MCF-7) cell line (American Type Culture Collection, Rockville, MD, USA).

Chemistry:

4-Aminopyrimido [4, 5-b] quinoline (3) Method (A): A solution of compound 2 (1.69 g, 0.001 mol) in formamide (30 ml) was refluxed for 5 h. The reaction mixture was cooled and then poured onto cold water, and the obtained solid was crystallized from dioxane to give compound 3. Yield 42 % (0.709 g), m.p. > 300 ^{0}C.

Method (B): A mixture of compound 18 (0.005 mol) and ammonia solution 35 % (10 mL) in absolute ethanol (15 mL) was heated at reflux temperature for 12 h. The solvent was evaporated under reduced pressure and the solid obtained was triturated with ice-water, filtered, washed with water, dried and crystallized from ethanol. Yield 80 %, m.p. > 300 ^{0}C.

^{1}HNMR (300MHz, [D_6] DMSO): δ = 4.65 (br.s, 2H, NH_2), 6.82 (m, 2H, C_6-H, C_7-H), 7.00 (m, 2H, C_8-H, C_9-H), 7.84 (s, 1H, C_5-H), 9.07 (s, 1H, C_2-H).). ^{13}C NMR (300MHz, [D_6] DMSO): δ= 121.6, 127.2, 128.4, 128.6, 129.1, 130.2, 136.7, 147.1, 156.9, 157.8, 160.1 (C-2).MS (m/z): 196.07 (22, M^+), 197 (28, M^{+1}). IR (KBr, ν, cm^{-1}): 3451(NH), 3062 (CH arom.). Anal. Calc. For $C_{11}H_8N_4$: C, 67.34; H, 4.11; N, 28.55. Found. C, 67.28; H, 4.07; N, 28.71.

2-Acetylaminoquinoline-3-carbonitrile (4): A solution of compound 2 (1.69 g, 0.001 mol) in acetic anhydride (20 ml) was refluxed for 5 h, the reaction mixture was then concentrated, and the solid separated was crystallized from ethanol to give compound 4. This procedure differ from the reported one [24] to increase yield of the produced compound. Yield 70%, m.p. 116-118°C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 1.97 (s, 3H, CH_3), 7.85 (t,1H, J = 8.0 , C_6-H), 8.03 (t, 1H, J = 8.0 , C_7-H), 8.17 (d, 1H, J = 8.0 , C_5-H), 8.24 (d, 1H, J = 8.0 , C_8-H), 8.60 (s, 1H, C_4-H), 10.28 (br.s, 1H, NH, D_2O exchangable). MS (m/z): 211.21 (51, M^+), 212 (17, M^{+1}). IR (KBr, ν, cm^{-1}): 3230(NH), 3050 (CH arom.), 2952, 2873 (CH aliph.), 2211 (C≡N), 1712 (C=O). Anal. Calc. For $C_{12}H_9N_3O$: C, 68.24; H, 4.29; N, 19.89. Found. C, 68.11; H, 4.43; N, 19.86.

2-(N, N-diacetylamino) quinoline-3-carbonitrile (5): A solution of compound 2 (1.69 g, 0.001 mol) in acetic anhydride (20 ml) was refluxed for 10 h, the reaction mixture was then concentrated and the solid separated was crystallized from ethanol to give compound 5. Yield 60%, m.p. 149-150°C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 2.28 (2s, 6H, $2CH_3$), 7.84 (t, 1H, J = 8.0 , C_6-H), 8.01 (t, 1H, J = 8.0 , C_7-H), 8.13 (d, 1H, J = 8.0 , C_5-H), 8.12 (d, 1H, J = 8.0 , C_8-H), 8.47 (s, 1H, C_4-H). MS (m/z): 211.21 (51, M^+), 212 (17, M^{+1}). IR (KBr, ν, cm^{-1}): 3067 (CH arom.), 2958, 2880 (CH aliph.), 2215 (C≡N), 1720 (C=O). Anal. Calc. For $C_{14}H_{11}N_3O_2$: C, 66.40; H, 4.38; N, 16.59. Found. C, 66.15; H, 4.53; N, 16.18.

2-Methylpyrimido [4, 5-b]quinolin-4(3H)-one (6) Method (A): A solution of compound 2 (1.69 g, 0.001 mol) in acetic anhydride (20 ml) was refluxed for 20 h, the reaction mixture was then concentrated, the solid separated was crystallized from ethanol to give compound 6 in high yield (85%) m.p. > 300 °C . This procedure differ from the reported one [25] to increase yield of the produced compound

Method (B): Acetyl chloride (0.005 mol) was added to a solution of 2 (1.69 g, 0.001 mol) in dry pyridine (30 ml) and the mixture was refluxed on a water bath for 3 hr., then left to cool to room temperature and poured into ice cold water and neutralized by diluted hydrochloric acid for

complete precipitation. The separated material was collected by filtration, washed with water, dried well and crystallized from acetic acid to yield compound 6 (65%), m.p. > 300 °C. .this procedure differ from the reported one [25] to increase yield of the produced compound

Yield 85 %, M. p. > 300 ^{0}C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 2.33 (s, 3H, CH_3), 7.36 (t, 1H, J = 7.0, C_7-H), 7.62 (t, 1H, J = 7.0, C_8-H), 7.80 (d, 1H, J = 8.0, C6-H), 7.83(br.s, 1H, NH, D_2O exchangable). 8.11 (d, 1H, J = 8.0, C_9-H), 8.55 (s, 1H, C_5-H). ^{13}C NMR (300MHz, [D_6] DMSO): δ= 25.2 (CH_3) 123.1, 127.8, 128.4, 129.2,130, 132.4, 139.7, 147, 154.3, 161.8, 169.5.MS (m/z): 211.22 (18, M^+), 212 (9, M^{+1}). IR (KBr, ν, cm^{-1}): 3422 (NH), 1703 (C=O). Anal. Calc. For $C_{12}H_9N_3O$: C, 68.24; H, 4.29; N, 19.89. Found. C, 68.15; H, 4.17; N, 19.75.

General procedure for synthesis of 2-functionalized 4-aminopyrimido [4, 5-b] quinoline 9a and 9b: A mixture of **2** (1.69 g, 0.001 mol) and urea (0.6 g, 0.005 mol) or thiourea (0.76 g, 0.005 mol) in absolute ethanol (20 ml) containing sodium ethoxide (0.68 g, 0.005 mol) was refluxed for 6 hr. The reaction mixture was left to cool to room temperature, then poured into ice cold water (50 ml) and neutralized with dilute hydrochloric acid 1%; the separated material was filtered off and crystallized from ethanol to give compounds 9a and 9b respectively, this a modified procedure to another reported one [25].

4-Aminopyrimido[4,5-b]quinolin-2(1H)-one(9a): Yield 75 %, M. p. > 300 ^{0}C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 5.93(s, 2H, NH_2, D_2O exchangeable), 7.51 (t, 1H, J = 7.0, C_8-H), 7.70 (m, 2H, C_6-H, C_7-H), 7.83 (d, 1H, J = 8.0, C_9-H), 8.22 (br.s, H, NH, D_2O exchangeable), 8.82 (s, 1H, C_5-H). ^{13}C NMR (DMSO-d6) δ: 116.5, 121.7, 122.3, 125.7, 127, 129.2, 132.3, 146.6, 148.4, 156.3, 164.2. MS (m/z): 212.21 (34, M^+), 213 (15, M^{+1}). IR (KBr, ν, cm^{-1}): 3375, 3165 (NH_2, NH), 1616 (C=O). Anal. Calc. For $C_{11}H_8N_4O$: C, 62.26; H, 3.80; N, 26.40. Found. C, 62.27; H, 3.92; N, 26.20.

4-Aminopyrimido [4, 5-b] quinoline-2(1H)-thione (9b): Yield 80 %, M. p. > 300 ^{0}C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 5.96 (s, 2H, NH_2, D_2O exchangeable), 7.33 (t, 1H, C_8-H), 7.43 (m, 2H, C_6-H, C_7-H), 7.89 (d, 1H, C_9-H), 8.15 (br.s, 2H, NH_2, D_2O exchangeable), 8.25 (s, 1H, C_5-H). MS (m/z): 228.27 (24, M^+), 229 (41, M^{+1}). IR (KBr, ν, cm^{-1}): 3395, 3228 (NH_2, NH), 1263 (C=S). Anal. Calc. For $C_{11}H_8N_4S$: C, 57.88; H, 3.53; N, 24.54. Found. C, 57.85; H, 3.67; N, 24.61.

2-(N-formylamino) quinoline – 3 - carbonitrile (10): An equimolar amount of **2** (1.69 g, 0.001 mol) and formic acid (0.005 mol) in absolute ethanol (30 ml) was refluxed for 2 hr. The reaction mixture was then concentrated and left to cool overnight to room temperature for complete precipitation. The precipitated solid was filtered off, dried well and recrystallized from aqueous ethanol to afford compound 10. Yield 62 %, M. p.202-203 ^{0}C. MS (m/z): 197.19 (48, M^+). IR (KBr, ν, cm^{-1}): 3225 (NH), 2215 (CN), 1689 (C=O), 1577 (C=N). Anal. Calc. For $C_{11}H_7N_3O$: C, 67.00; H, 3.58; N, 21.31. Found. C, 67.09; H, 3.51; N, 21.30.

Pyrimido [4, 5-b] quinolin-4(3H)-one (12): Solution of **10** (1.97 g, 0.002 mol) in potassium carbonate (10 %, 10 mL) and hydrogen peroxide (30 %, 5 mL) was refluxed for 1 hr. The reaction mixture was left to cool at room temperature for complete precipitation. The precipitated solid was collected by filtration and recrystallized from aqueous ethanol to produce compound 12. Yield 77 %, M.p.202-203^{0}C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 6.87-7.80 (m, 4H, C_8-H, C6-H, C_7-H, C_9-H), 7.90 (br.s, 1H, NH, D_2O exchangeable), 8.09 (s, 1H, C_2-H), 8.11 (s, 1H, C_5-H). ^{13}C NMR (DMSO-d6) δ: 122.1, 127.9, 128.7, 129.1,130, 132.3, 139.1, 145.7, 147.2, 161, 168.5. MS (m/z): 197.19 (48, M^+). IR (KBr, ν, cm^{-1}): 3140 (NH), 1701 (C=O), 3072 (CH arom.). Anal. Calc. For $C_{11}H_7N_3O$: C, 67.00; H, 3.58; N, 21.31. Found. C, 67.23; H, 3.23; N, 21.42.

N'-(3-cyanoquinolin- 2 - yl) - N, N - dimethyl formamidine (13): A solution of **2** (1.69 g, 0.001 mol) in dry dioxane (20 ml) and DMF-DMA (0.005 mol) was refluxed for 4 hr. and the reaction mixture was then cooled to room temperature and poured into ice/cold water to complete precipitation. The solid was filtered off and recrystallized from ethanol to give compound 13.

Yield 82 %, M.p. 214-215 0C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 3.2 (s, 6H, $2CH_3$), 7.32-7.80(m, 4H, Ar-H), 8.2 (s, 1H, N=CH), 8.73 (s, 1H, C4-H). MS (m/z): 224.26 (12, M^+). IR (KBr, ν, cm^{-1}): 2216 (CN), 1623 (C=N), 3099 (CH arom.), 2885 (CH aliph.). Anal. Calc. For $C_{13}H_{12}N_4$: C, 69.62; H, 5.39; N, 24.98. Found. C, 69.77; H, 5.41; N, 24.90.

3(4H)-Amino-4-iminopyrimido [4, 5-b]quinolin-(15): Method (A): A mixture of 13 (2.24 g, 0.005 mol) and hydrazine hydrate 98 % (0.5 ml, 0.005 mol) in absolute ethanol (30 ml) was refluxed for 4 hr. and the reaction mixture was left at room temperature overnight and then poured into ice/cold water to complete precipitation. The product was filtered off and recrystallized from dry benzene to give compound 15. Yield 63 %, M. p.225-227 ^{0}C.

Method (B): A mixture of compound 18 (2.25 g, 0.005 mol) and hydrazine hydrate 98 % (0.5 ml, 0.05 mol) in absolute ethanol (15 mL) was heated at reflux temperature for 12 h. The reaction mixture was cooled and the precipitated solid was collected by filtration, washed with water, dried and crystallized from dioxane to give compound 15. Yield 85 %, M. p.225-227 ^{0}C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 5.4 (s, 2H, NH_2, D_2O exchangeable), 7.21-7.60 (m, 4H, Ar-H), 7.65 (s, 1H, C_2-H pyrimidine), 8.2 (s, 1H, NH, D_2O exchangeable), 8.89 (s, 1H, C_5-H). ^{13}C NMR (300MHz, [D_6] DMSO): δ= 118.8, 127, 127.2, 128.5, 129, 130, 134.7, 149.8, 163.2, 164.1, 167.9. MS (m/z): 224.26 (12, M^+). IR (KBr, ν, cm^{-1}): 3421-3360 (NH_2), 3148 (NH), 3106 (CH arom.). Anal. Calc. For $C_{11}H_9N_5$: C, 62.55; H, 4.29; N, 33.16. Found. C, 62.66; H, 4.31; N, 33.32.

General procedure for synthesis of 4-Imino-3-phenyl-3, 4-dihydropyrimido [4, 5-b]quinoline one / thione derivatives 16a and 16b: An equimolar mixture of 2 (1.69 g, 0.001 mol) and phenylisocyanate (1.35 g, 0.005 mol) or phenyl–isothiocyanate (1.19g, 0.005mol) in dimethyl formamide (30 mL) in the presence of a few drops of triethylamine (4 drops) was refluxed for 10 hr. The reaction mixture was left to cool and poured into cold water for complete precipitation. The separated solid was filtered off, washed with water, dried well and recrystallized from ethanol to give compounds 16a, 16b.

4-Imino-3-phenyl-3,4-dihydropyrimido [4, 5-b] quinolin-2(1H)-one(16a): Yield 55 %, M. p. 228-229 ^{0}C. ^{1}HNMR (300MHz, [D_6] $CDCl_3$): δ = 6.92–7.98 (m, 9H, Ar–H, C5–H, 2NH). MS (m/z): 288.30 (19, M^+), 289 (3, M^{+1}). IR (KBr, ν, cm^{-1}): 3165, 3099 (2NH), 1640 (C=N), 1705 (C=O).Anal. Calc. For $C_{17}H_{12}N_4O$: C, 70.82; H, 4.20; N, 19.43. Found. C, 70.99; H, 4.18; N, 19.40.

4-Imino-3-phenyl-3,4-dihydropyrimido [4, 5-b] quinoline-2(1H)-thione (16b): Yield 58 %, M. p. 207-209 0C. ^{1}HNMR (300MHz, [D_6] DMSO-D_2O): δ= 6.87–8.02 (m, 9H, Ar–H, C5-H, 2NH). MS (m/z): 304.37 (21, M^+), 213 (7, M^{+1}). IR (KBr, ν, cm^{-1}): 3170, 3131 (2NH), 1632 (C=N), 1346 (C=S). Anal. Calc. For $C_{17}H_{12}N_4S$: C, 67.08; H, 3.97; N, 18.41. Found. C, 67.15; H, 3.73; N, 18.29.

2-(Chloromethyl) pyrimido [4, 5-b] quinolin-4(3H)-one (17): A mixture of compound 2 (1.69 g, 0.001 mol) and chloroacetyl chloride (1.12 g, 0.01 mol) in dimethyl formamide (20 ml) was refluxed for 20 h. The reaction mixture was cooled and then poured onto cold water and the solid obtained was crystallized from dioxane to give 17. Yield 58 %, M. p. >300 ^{0}C. ^{1}HNMR (300MHz, [D_6] DMSO- D_2O): δ = 4.9 (s, 2H, CH_2Cl), 7.08-7.95 (m, 4H, C_8-H, C_6-H, C_7-H, C_9-H), 8.02 (s, 1H, C_5-H), 8.10 (s, 1H, NH). MS (m/z): 245 (37, M^+), 247(12, M^{+2}). IR (KBr, ν, cm^{-1}): 3226 (NH), 2958, 2878, 1670(C=O), 1594(C=N), Anal. Calc. For $C_{12}H_8ClN_3O$: C, 58.67; H, 3.28; N, 17.10. Found. C, 58.57; H, 3.15; N, 17.15.

Ethyl N-3-cyanoquinolin-2-ylformimidate (18): A mixture of compound 2 (1.69 g, 0.001 mol) and triethyl orthoformate (10 mL) was heated at reflux temperature for 10–12 h. Excess reagent was evaporated under reduced pressure, and the solid obtained was triturated with ice water, filtered, washed with water, dried and crystallized from ethanol to afford compound 18. Yield 90 %, M. p. 173-174 0C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 1.2 (t, 3H, CH_3, J= 7.3Hz), 4.3 (q, 2H, CH_2 ethyl, J= 7.3 Hz), 7.01-7.80(m, 5H, Ar-H), 8.32 (s, 1H, N=CH), 8.56 (s, 1H, C_4-H). MS (m/z): 225.25 (57, M^+).

IR (KBr, ν, cm^{-1}): 3063(CH arom.), 2950, 2891 (CH aliph.), 2198(C≡N). Anal. Calc. For $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66. Found. C, 69.34; H, 5.03; N, 18.70.

1-Benzoyl-3-(3-cyanoquinolin - 2 - yl) thiourea (19): A mixture of 2 (1.69 g, 0.001 mol) and benzoylisothiocyanate (0.005 mol) in dimethylformamide (30 mL) containing a catalytic amount of triethylamine (4 drops) was refluxed for 6 hr. and left to cool to room temperature. The reaction mixture was poured into cold water for complete precipitation, and then filtered off, washed with water dried well and recrystallized from aqueous methanol to give compound 19. Yield 65 %, M. p.194-195 ^{0}C. MS (m/z): 332.38 (23, M^+). IR (KBr, ν, cm^{-1}): 3184, 3103(2NH), 3054(CH arom.), 2212(C≡N), 1677 (C=O), 1346(C=S). Anal. Calc. For $C_{18}H_{12}N_4OS$: C, 65.04; H, 3.64; N, 16.86. Found. C, 65.17; H, 3.51; N, 16.80.

Benzoylamino - 1, 2 - dihydropyrimido[4, 5 b] quinoline 2–(1H)thione (21): Method A: To a solution of 19 (3.33 g, 0.005 mol) in dimethylformamide was added few drops of triethylamine. The reaction mixture was refluxed 6 hr., then left to cool, the solids filtered off, washed with water, dried well and recrystallized from ethanol to give compound 21. Yield 35 %, M. p.236-237 ^{0}C.

Method B: An equimolar mixture of 2 (1.69 g, 0.001 mol) and benzoylisothiocyanate (0.005 mol) was refluxed for 10 hr. in dimethylformamide (30 mL) containing four drops of triethylamine. The reaction mixture was left to cool and poured into cold water for complete precipitation. The separated solid was filtered off, washed with water, dried well and recrystallized from ethanol to give compound 21. Yield 62 %, M. p.236-237 ^{0}C.

^{13}C NMR (300MHz, [D_6] DMSO): δ = 116.4, 121.5, 122.2, 125.4, 126.8,127.5(2C), 129(2C), 129.2, 132.2, 133, 134.4, 148.3, 161.2, 164, 169.3, 183.4. MS (m/z): 332.38 (18, M^+). IR (KBr, ν, cm^{-1}): 3180 (NH), 3117 (NH), 1668 (C=O), 1603 (C=N), 1360 (C=S). Anal. Calc. For $C_{18}H_{12}N_4OS$: C, 65.04; H, 3.64; N, 16.86. Found. C, 65.17; H, 3.51; N, 16.80.

Pyrimido [4, 5-b] quinoline-2, 4(1H,3H)-dithione (24): To a solution of 2 (1.69 g, 0.001 mol) in dry pyridine (30 mL) carbon disulphide (0.005 mol) was added and the reaction mixture was refluxed on a water bath for 6 hr., then left to cool to room temperature, poured into cold water and neutralized with diluted hydrochloric acid to complete precipitation. The solid obtained was filtered off, washed with water, dried well and recrystallized from methanol to give compound 24. Yield 75 %, M. p.241-242^{0}C. ^{1}HNMR (300MHz, [D_6] DMSO): δ = 5.75 (s, 1H, NH, D_2O exchangeable);7.33 (t, 1H, J = 7.1 , C8-H), 7.45 (d, 1H, J = 8.5 , C6-H), 7.71 (t, 1H, J = 8.5 , C7-H), 8.03 (d, 1H, J = 8.3 , C9-H), 8.86 (s, 1H, C5-H), 9.23 (br.s, 1H, NH), 9.62 (br.s, 1H, NH , D_2O exchangeable). ^{13}C NMR (300MHz, [D_6] DMSO): δ= 121.2, 122.3, 125.3, 125.6,126.8, 129, 136.2, 149.3, 168, 171.3, 180.4. MS (m/z): 245.32 (29, M^+). IR (KBr, ν, cm^{-1}): 3421 (NH), 1632 (C=N), 1339 (C=S). Anal. Calc. For $C_{11}H_7N_3S_2$: C, 53.85; H, 2.88; N, 17.13. Found. C, 53.77; H, 2.90; N, 17.06.

ACKNOWLEDGMENTS: The authors would like to express their sincere thanks to Dr. Mohammad sadek, Pharmacology Department, Faculty of Pharmacy Al-Azher University, Cairo, Egypt, for carrying out the cytotoxicity testing for starting compound. And I would like to thanks all members of Pharmacology Unit at the National Cancer Institute (NCI), Cairo University, for performing the cytotoxicity testing

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How to cite this article:
El-Gamal KM: Synthesis and anticancer screening of heterocyclic compounds bearing pyrimido [4, 5-b] quinoline moiety. Int J Pharm Sci Res 2017; 8(2): 570-81.doi: 10.13040/IJPSR.0975-8232.8(2).570-81.