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Utility of polyhydroquinoline as synthons for anticancer fused pyrimidines

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ABSTRACT

Polyhydroquinoline **(1)** interacted with DMF-DMA to yield the corresponding aza-enamine derivative **(2)**. Compound **(2)** reacted with a series of aromatic amines to afford the new fused pyridopyrimidines **(5a-d)**. On the other hand, reaction of **(2)** with hydrazine hydrate and hydroxylamine hydrochloride yielded the pyrimidine derivatives **(7)** and **(8)**, respectively. Refluxing of **(2)** with urea derivative in acetic acid yielded the final product **(10a,b)**. Studying the behavior of **(2)** towards active methylene reagent afforded the adducts **(11)** and **(12)**, respectively. The in vivo antitumor activity of compounds **(5a-c)**, **(8)** and **(10a)** was evaluated against liver cancer cells (HEPG2), using Doxorubicine as a reference drug. Compound **(5b)** showed the highest potency mean while compounds **(5c)** and **(8)** showed limited activity against liver carcinoma cells (HEPG2). Also, the in vivo antitumor activity, compound **(5b)** exhibited high cytotoxic activity against breast cancer cells (MCF7).

KEYWORDS

Quinoline;
Pyridopyrimidines;
Dimorth rearrangement;
Anticancer agents.

INTRODUCTION

Hepatic cancer is a cancer that originates in the liver this type of cancer varies widely in incidence throughout the world, with rising incidence in Egypt. Incidence of the most common type of liver cancer, hepatocellular carcinoma (HCC), have doubled in Egypt over the past 12 years and this put Egypt into thaintermediate category of prevalence. Heterocyclic nucleus imparts an important role in medicinal chemistry and serves as a key template for the development of various therapeutic agents. Synthetic studies of fused pyrimidine have been reported extensively because of their structural diversity and association with a wide spectrum of biological activity.

Pyrimidine is the parent hetero ring of a very important group of compounds that are extensively studied due to their occurrences in living systems[1,2]. Compounds containing pyrimidine ring have been reported as antibacterial and antifungal agents, as well as those exhibiting anti-HIV activity[3-6]. A variety of condensed pyrimidines, especially those with primary amino or substituted amino function at 4-position have been synthesized and have been evaluated as antifolate, antimalarial activities[7-9]. In addition to the above mentioned activities, fused pyrimidines possessing anti-inflammatory, anti-analgesic, anti-tumor and anti-infective activities have also been reported[10-13].

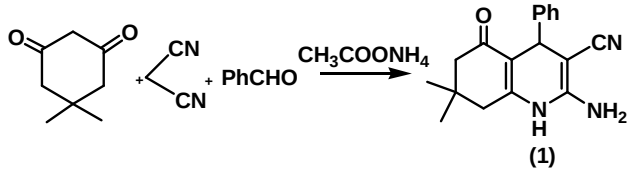
Full Paper

Continuation to our previous efforts[14-16] in search for potential anticancer molecules, we have synthesized new derivatives of fused pyridopyrimidines depending on the easily synthesized aza-examine **(2)** from polyhydroquinoline **(1)** as starting material. Some of the newly synthesized fused pyrimidines were evaluated as anticancer agents against hepatocellular, and breast carcinoma cell lines.

RESULTS AND DISCUSSION

Chemistry

4-Phen-yl-3-cyano-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline **(1)** was synthesized, according to scheme 1, *via* one pot three-component reaction as reported previously[17].



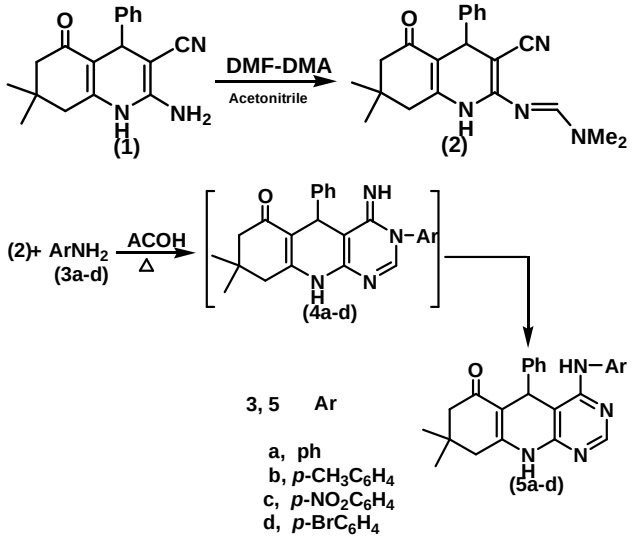
Scheme 1

Compound **(1)** was allowed to react with N,N-dimethylformamide dimethylacetal; DMF-DMA to form the corresponding aza-enamine derivative **(2)** which was utilized as a precursor for synthesis a variety of fused pyridopyrimidines. The structure of **(2)** was confirmed with different spectroscopic data (*cf.* experimental section). The aza-enamine derivative **(2)** has interacted with different aromatic amines in acetic acid to afford the corresponding pyrimido[4,5-*b*]quinolinones **(5a-d)** (Scheme 2).

The structure of compounds **(5a-d)** was established by the analytical and spectroscopic data. IR spectrum of compound **(5a)** which was taken as example exhibited the disappearance of the CN band and the existence of the NH band at ν 3080 cm^{-1}.

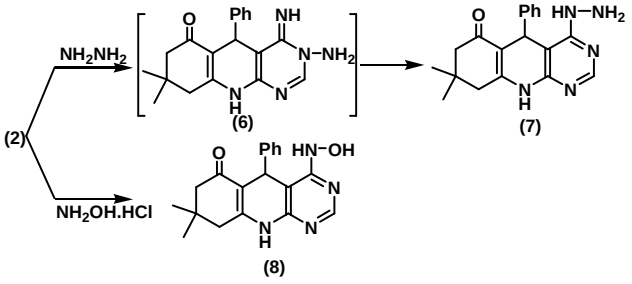
The ^{1}H-NMR spectrum of **(5a)** revealed two singlets at δ 0.90 and 1.07 assigned to the two methyl protons, two singlet at δ, ppm: 2.37, 2.50 for the two methylene groups, two multipltes at δ 7.02-7.26 and 7.29-7.56 for the aromatic protons and NH group, singlet at δ 8.29 D_2O exchanged for the other NH-Ph group and singlet at δ 8.69 ppm consistent with the proton of CH at the pyrimidine ring. All the other data were in accordance with the suggested structure (*cf.* experimental section).

The formation of **(5a-d)** was occurred *via* nucleophilic substitutions on the aza-methylidene carbon followed by ring closer to yield the imino derivatives **(4)** at first which underwent a Dimorth rearrangement[18,19] to form the thermodynamically more stable final products **(5)**. (Scheme 2)



Scheme 2

Interaction of **(2)** with hydrazine hydrate and hydroxylamine hydrochloride under the same previous conditions yielded 4-hydrazinyl dihydropyrimidoquinoline **(7)** and 4-hydroxyamino dihydropyrimidoquinoline **(8)**, respectively. The reaction was also proceeded through the intermediate **(6)** which rearranged under the reaction condition to form the more stable adduct **(7)** (Scheme 3).

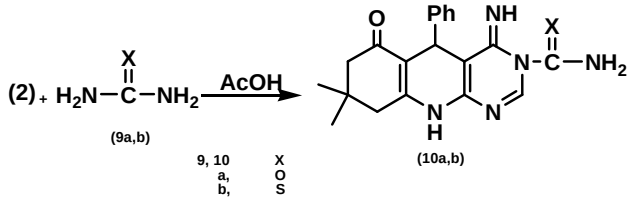


Scheme 3

IR spectrum of compound **(7)** exhibited strong absorption bands at 3442, 3068 cm^{-1} attributed to the NH_2, and NH groups. The ^{1}H-NMR spectra revealed three D_2O exchangeable signals at δ, ppm: 3.36 singlet for NH_2 protons, 7.64, and 9.80 ppm for the 2NH pro-

tons. This is in addition a singlet at δ, ppm: 9.40 ppm for the pyrimidine CH proton. All the analytical and spectroscopic data were in accordance with the structures of **(7)** and **(8)** (*cf*. experimental section).

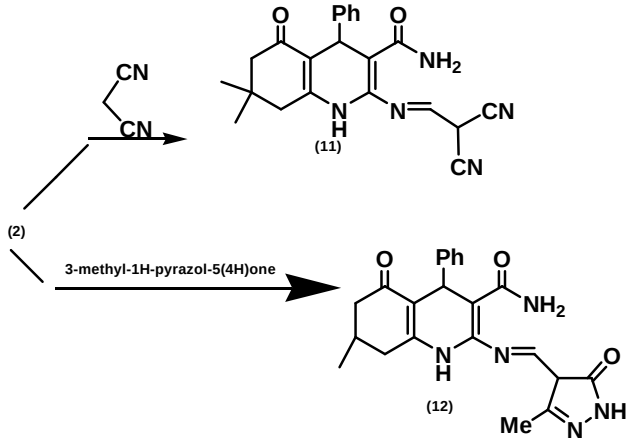
Refluxing of **(2)** with urea and thiourea **(9a,b)** in acetic acid yielded the corresponding products **(10a,b)** respectively. The reaction took place *via* nucleophilic substitution on the methylidene carbon followed by ring closer to furnish the final adduct **(10a,b)** (Scheme 4).



Scheme 4

The ^{1}H-NMR of compound **(10a,b)** revealed apparent signals at δ, ppm: 3.50 and 7.83 ppm assigned to exchangeable protons of NH_2 and NH.

Studying the behavior of compound **(2)** towards active methylene reagents was also carried out and represented in (Scheme 5). Compound **(2)** was allowed to react with malononitrile, and/or 3-methyl-1*H*-pyrazol-5(4H)one respectively. The reaction in this case showed aza-enamine substitution with hydrolysis of the nitrile group to furnish the isolated adducts **(11)** and **(12)** respectively. Trials to cyclize the isolated products **(11)** and **(12)** were failed.



Scheme 5

Bioactivity

Antitumor activity

Evaluation of anticancer activity of compounds **(5a-c)**, **(8)** and **(10a)** was performed at the National Cancer Institute (NCI), Cairo Egypt. The tested compounds were evaluated for cytotoxicity against liver and breast carcinoma cell lines, HEPG2 and MCF7 respectively. Different concentrations of the investigated compounds were added to the cell monolayer of tumor. A 48h continuous drug exposure is used to estimate growth[20]. TABLES 1&2 indicate the cytotoxic activities of both the reference drug and the investigated compounds against HEPG2. Figures (1,2,3 and 4) represented also the LC_{50}

TABLE 1 : Drug cytotoxicity against HEPG2

Compound	Conc. µg/ml	HEPG2	LC_{50}
	0.0	1.0	
	5.0	0.331906	
Doxorubicine	12.5	0.211934	3.73
	25.0	0.188967	
	50	0.262134	

TABLE 2 : The cytotoxic activity of the investigated compounds against HEPG2

Compound	Conc. µg/ml	HEPG2	LC_{50}
	0.0	1.0	
	5.0	0.731322	
5a	12.5	0.349597	9.68
	25.0	0.144304	
	50	0.204569	
	0.0	1.0	
	5.0	0.282965	
5b	12.5	0.065138	3.2
	25.0	0.108522	
	50	0.202771	
	0.0	1.0	
	5.0	0.816335	
5c	12.5	0.187937	15.1
	25.0	0.091013	
	50	0.155481	
	0.0	1.0	
	5.0	0.901425	
8	12.5	0.268129	17.8
	25.0	0.108971	
	50	0.184478	
	0.0	1.0	
	5.0	0.682594	
10a	12.5	0.147336	11.7
	25.0	0.076290	
	50	0.161614	

of both the Doxorubicine drug and the tested compounds. It was found that compound **(5b)** is more active than the comparative drug (LC_{50} = 3.2 µg/ml) as shown in Figure 3. It is also noticed that compound **(5a)** was of higher cytotoxicity than that of **(10a)**, **(5c)**, and **(8)**, since their LC_{50} are 9.68, 11.7, 15.1, and 17.8 µg/ml respectively. All these values are compared with the LC_{50} value of the reference Doxorubicine against HEPG2.

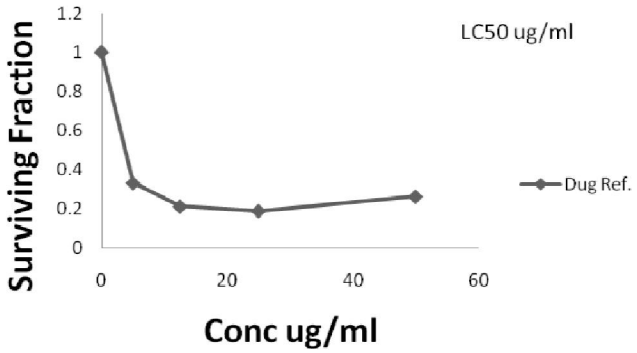


Figure 1 : Cytotoxic activity of the standard doxorubicine against HEPG2

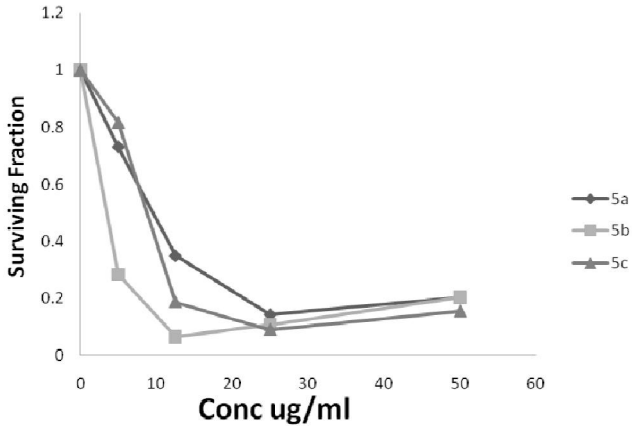


Figure 2 : Cytotoxic activity of compounds (5a-c) against HEPG2

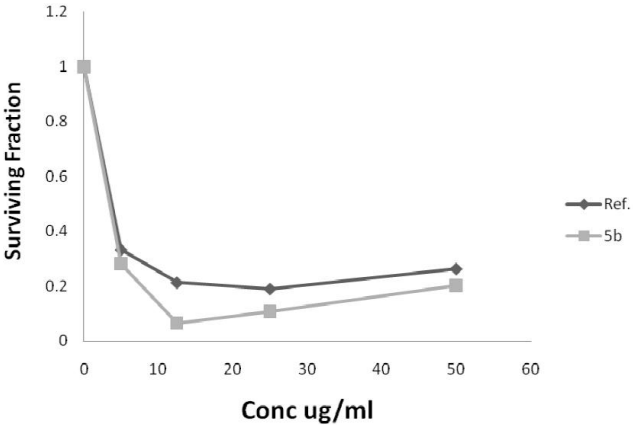


Figure 3 : Cytotoxic activity of compound (5b) against HEPG2 compared with the reference drug

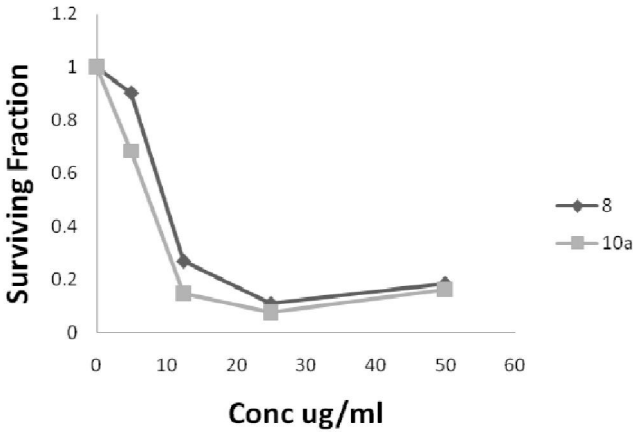


Figure 4 : Cytotoxic activity of compounds (8) and (10a) against HEPG2

Evaluation of anticancer activity of the above mentioned compounds against breast carcinoma (MCF7), the derivative **(5b)** showed good anticancer activity. TABLES 3 and 4 indicate the cytotoxic activity of both

TABLE 3 : Drug cytotoxicity against MCF7

Compound	Conc. µg/ml	MCF7	LC_{50}
Doxorubicine	0.0	1.0	2.97
	5.0	0.194273	
	12.5	0.171715	
	25.0	0.185526	
	50	0.201330	

TABLE 4 : The cytotoxic activity of the investigated compound against MCF7

Compound	Conc. µg/ml	MCF7	LC_{50}
5b	0.0	1.0	4.04
	5.0	0.396464	
	12.5	0.223500	
	25.0	0.147040	
	50	0.176447	

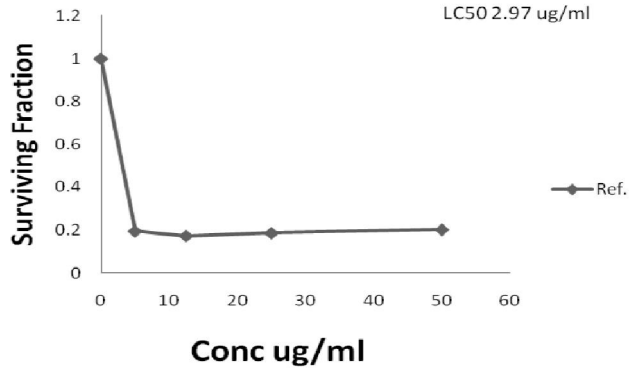


Figure 5 : Cytotoxic activity of the standard doxorubicine against MCF7

the comparative drug and compound **(5b)**. In this case compound **(5b)** showed also high anticancer activity against breast carcinoma cells when compared with the reference drug (LC_{50} = 4.04). Figures 5 and 6 represented the cytotoxic activity of the. Doxorubicine reference drug and **(5b)** derivative against breast cancer cell. Compounds under investigation showed no cytotoxic activity when examined against lung and colon cancer cells.

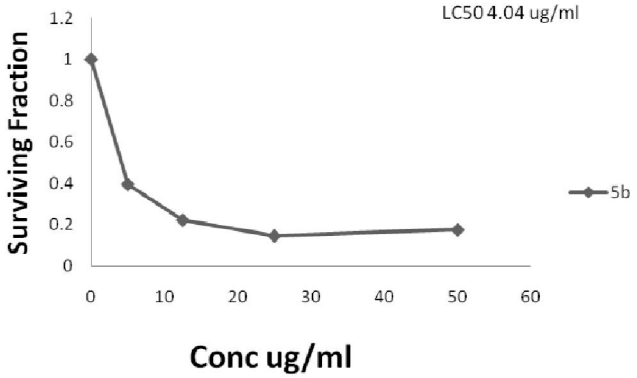


Figure 6 : Cytotoxic activity of compound (5b) against MCF7

EXPERIMENTAL

Melting points were determined on an electrothermal apparatus (Buchi 535, Switzerland) in an open capillary tube and are uncorrected. IR spectra expressed in (cm^{-1}) were recorded in KBr pellets on a PA-9721 IR spectrophotometer. ^{1}H-NMR & ^{13}C-NMR spectra were obtained on a Varian EM-390 (270 and 500 MHz) spectrometer in DMSO-d_6 as solvent, using TMS as internal reference and chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment. All reactions were monitored by thin layer chromatography, carried out on 0.2 mm silica gel 60 F-254 (Merck) plates using UV light (254 and 366 nm) for detection. Column chromatography was carried out on a Baker silica gel powder (60-200 mesh). Elemental analyses were carried out by the Microanalytical unit at the National Research Centre, Giza, Egypt. Antitumor activity was evaluated by the National Cancer Institute, Cancer Biology Department, Cairo University, Egypt.

Synthesis of aza-enamine (2)

General procedure

Synthesis of aza-enamine derivative **(2)** has been carried out according to the previously published procedure[17].

(E)-*N*'-(3-cyano-7,7-dimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquino line-2-yl)-N,N-dimethylformamidine (2)

White crystals, (EtOH) yield (85%); mp 228-230°C, Ms. m/z: 348 [M^+, 60%]. IR (KBr) ν cm^{-1}: 3086 (NH); 2957 (C-H, aliphatic); 2232 (CN), 1695 (C=O). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 1.03, 1.10 (2s, 6H, $2CH_3$); 2.20, 2.47 (2s, 4H, $2CH_2$); 3.06, 3.09 (2s, 6H, N-Me_2); 4.51 (s, 1H, -CH); 7.24-7.26 (m, 6H, 5 aromatic protons and NH); 8.01 (s, 1H, N=CH). Anal. Calcd. For Molecular Formula $C_{21}H_{24}N_4O$: C, 72.39%; H, 6.94%; N, 16.08%; Found: C, 72.28%; H, 6.72%; N, 15.96%.

Synthesis of (5a-d)

General procedure

To a solution of aza-enamine derivative **(2)** (0.01 mol) in acetic acid (30 ml), an equivalent amount of aromatic amines (0.01 mol) were added. The reaction mixture was heated under reflux for 8h until all stating materials disappeared that indicated by TLC, then poured into ice-water. The solid product was crystallized from suitable solvent.

8,8-dimethyl-5-phenyl-4-(phenylamino)-8,9-dihydropyrimido[4,5-*b*] quinolin-6(5*H*,7*H*,10*H*)-one (5a)

Yellowish crystals, (benzene) yield (75%), mp. 182-183°C, (EtOH), Ms. m/z: 396 [M^+, 50%]. IR (KBr) ν cm^{-1}: 3080 (NH); 2938 (C-H, aliphatic); 1689 (C=O), 1640 (C=N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 0.90, 1.07 (2s, 6H, $2CH_3$); 2.37, 2.50 (2s, 4H, $2CH_2$); 5.46 (s, 1H, -CH); 7.02-7.26 (m, 5H, 5 aromatic protons), 7.29-7.56 (m, 6H, 6 aromatic protons and NH); 8.29 (s, 1H, NH exchangeable), 8.69 (s, 1H, pyrimidine CH). Anal. Calcd. For Molecular Formula $C_{25}H_{24}N_4O$: C, 75.73%; H, 6.10%; N, 14.13%; Found: C, 75.50%; H, 6.025%; N, 13.91%.

4-(*p*-toluidino)-8,8-dimethyl-5-phenyl-8,9-dihydropyrimido[4,5-*b*]quinolin -6(5*H*,7*H*,10*H*)-one (5b)

Yellowish white crystals, (EtOH), yield (65%); mp.

162 °C, Ms. m/z: 410 [M^+, 65%]. IR (KBr) ν cm^{-1}: 3020 (NH); 2953 (C-H, aliphatic); 1682 (C=O), 1643 (C=N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 0.93, 1.07 (2s, 6H, $2CH_3$); 2.10 (s, 3H, CH_3); 2.23, 2.36 (2s, 4H, $2CH_2$); 5.47 (s, 1H, -CH); 7.06-7.26 (m, 5H, 5 aromatic protons), 7.37-7.42 (m, 5H, 4 aromatic protons and NH); 8.28 (s, 1H, NH exchangeable), 8.61 (s, 1H, pyrimidine CH). Anal. Calcd. For Molecular Formula $C_{26}H_{26}N_4O$: C, 76.07%; H, 6.36%; N, 13.65%; Found: C, 76.19%; H, 6.17%; N, 13.36%.

8,8-dimethyl-4-(4-nitophenylamino)-5-phenyl-8,9-dihydropyrimido[4,5-*b*] quinolin-6(5*H*,7*H*,10*H*)-one (5c)

Yellowish crystals, (EtOH), yield (60%); mp. 172-174°C, Ms. m/z: 425 [M^+, 40%]. IR (KBr) ν cm^{-1}: 3047 (NH); 2957 (C-H, aliphatic); 1695 (C=O); 1642 (C=N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 0.95, 1.05 (2s, 6H, $2CH_3$); 2.25, 2.42 (2s, 4H, $2CH_2$); 4.71 (s, 1H, -CH); 7.11-7.22 (m, 5H, 5 aromatic protons), 7.80-8.19 (m, 5H, 4 aromatic protons and NH); 8.22 (s, 1H, NH exchangeable), 10.54 (s, 1H, pyrimidine CH). Anal. Calcd. For Molecular Formula $C_{25}H_{23}N_5O_2$: C, 70.57%; H, 5.45%; N, 16.46%; Found: C, 70.31%; H, 5.26%; N, 16.51

4-(bromophenylamino)-8,8-dimethyl-5-phenyl-8,9-dihydropyrimido[4,5-*b*] quinolin-6(5*H*,7*H*,10*H*)-one (5d)

Yellowish crystals, (EtOH), yield (70%); mp. 198-200°C, Ms. m/z: 475 [M^+, 45%]. IR (KBr) ν cm^{-1}: 3116 (NH); 2962 (C-H, aliphatic); 1696 (C=O), 1649 (C=N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 0.93, 1.07 (2s, 6H, $2CH_3$); 2.31, 2.37 (2s, 4H, $2CH_2$); 5.47 (s, 1H, -CH); 7.12-7.38 (m, 5H, 5 aromatic protons), 7.42-7.59 (m, 5H, 4 aromatic protons and NH); 8.33 (s, 1H, NH exchangeable), 8.82 (s, 1H, pyrimidine CH). ^{13}C-NMR (270 MHz, DMSO-d_6, TMS): 26.94 (C5), 29.12 (C2), 31.75 (C1), 32.45 ($2CH_3$), 50.53 (C3), 100.72 (C13), 114.67 (C11), 114.67 (C11), 115.49, 123.86, 127.41, 128.31 (C-Ph), 128.85, 131.69, 139.07, 143.55 (C, of *p*-bromo), 156.43 (C12), 159.11 (C8), 161.93 (C6), 164.49(C14), 196.42 (C=O). Anal. Calcd. For Molecular Formula $C_{25}H_{23}N_4OBr$: C, 63.16%; H, 4.88%; N, 11.79%; Found: C, 63.24%; H, 4.66%; N, 11.48%.

Reaction of aza-enamine (2) with hydrazine hydrate/hydroxyl amine

General procedure

Equimolar amounts of each of compound **(2)** (0.01 mol) and (0.01 mol) hydrazine hydrate or hydroxyl amine hydrochloride in dry acetic acid (25ml) were heated under reflux for 8hr. The solvent was evaporated under vacuum and the remaining residue was washed with n-hexane. After filtration, the solid product was collected and crystallized from suitable solvent.

4-hydrazinyl-8,8-dimethyl-5-phenyl-4-8,9-dihydropyrimido[4,5-*b*] quinolin-6(5*H*,7*H*,10*H*)-one (7)

Yellow crystals, (benzene, pet. Ether) yield (55%); mp. 132 °C, Ms. m/z: 335 [M^+, 36%]. IR (KBr) ν cm^{-1}: 3442 (NH_2), 3068 (NH); 2936 (C-H, aliphatic); 1687 (C=O), 1646 (C=N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 0.99, 1.08 (2s, 6H, $2CH_3$); 2.43, 2.50 (2s, 4H, $2CH_2$); 3.36 (s, 2H, NH_2, D_2O exchangeable), 5.21 (s, 1H, -CH); 7.24-7.30 (m, 5H, 5 aromatic protons), 8.80 (s, 1H, NH, D_2O exchangeable), 9.00 (s, 1H, pyrimidine CH), 9.80 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For Molecular Formula $C_{19}H_{21}N_5O$: C, 68.04%; H, 6.31%; N, 20.88%; Found: C, 68.01%; H, 6.30%; N, 20.85

4-(hydroxyamino)-8,8-dimethyl-5-phenyl-4-8,9-dihydropyrimido[4,5-*b*] quinolin-6(5*H*,7*H*,10*H*)-one (8)

Yellowish crystals, (EtOH), yield (65%); mp. 206 °C, Ms. m/z: 336 [M^+, 35%]. IR (KBr) ν cm^{-1}: 3427(OH), 3047 (NH); 2925 (C-H, aliphatic); 1642 (C=N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 0.90, 1.05 (2s, 6H, $2CH_3$); 2.31, 2.49 (2s, 4H, $2CH_2$); 4.71 (s, 1H, -CH); 4.90 (s, 1H, OH, D_2O exchangeable); 7.12-7.33 (m, 6H, 5 aromatic protons and NH), 8.08 (s, 1H, NH, D_2O exchangeable), 8.25 (s, 1H, pyrimidine CH). Anal. Calcd. For Molecular Formula $C_{19}H_{20}N_4O_2$: C, 67.84%; H, 5.99%; N, 16.66%; Found: C, 67.73%; H, 5.76%, 16.43%.

Reaction of aza-enamine (2) with urea/thiourea

General procedure

Equimolar amounts of each of compound **(2)** (0.01

mol) and urea or thiourea in glacial acetic acid (25ml) were heated under reflux for 14/11hr. The solvent was evaporated under vacuum and the remaining residue was washed with n-hexane. After filtration, the solid product was collected and crystallized from suitable solvent.

4-Imino-8,8-dimethyl-6-oxo-5-phenyl-5,6,7,8,9,10-hexahydro-4*H*-pyrimido[4,5-*b*]quinoline-3-carboxylic acid amide (10a)

Buff crystals, (benzene), yield (65%); mp. 110 °C, Ms. m/z: 363 [M^+, 50%]. IR (KBr) ν cm^{-1}: 3450(NH_2), 3057 (NH); 2922 (C-H, aliphatic); 1705 (C=O), 1665 (C=O), 1640 (C=N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 0.93, 1.05 (2s, 6H, $2CH_3$); 2.30, 2.42 (2s, 4H, $2CH_2$); 3.50 (s, 2H, NH_2, D_2O exchangeable), 5.12 (s, 1H, -CH); 7.18-7.44 (m, 5H, 5 aromatic protons), 7.83 (s, 1H, NH, D_2O exchangeable), 8.54 (s, 1H, pyrimidine CH). Anal. Calcd. For Molecular Formula $C_{20}H_{21}N_5O_2$: C, 66.10%%; H, 5.82%; N, 19.27%; Found: C, 66.08%; H, 5.80%, N, 19.25%.

4-Imino-8,8-dimethyl-6-oxo-5-phenyl-5,6,7,8,9,10-hexahydro-4*H*-pyrimido[4,5-*b*]quinoline-3-carbothioic acid amide (10b)

Yellowish crystals, (EtOH), yield (65%); mp. 156 °C, Ms. m/z: 379 [M^+, 35%]. IR (KBr) ν cm^{-1}: 3427(NH_2), 3047 (NH); 2925 (C-H, aliphatic); 1701 (C=O), 1642 (C=N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 0.90, 1.05 (2s, 6H, $2CH_3$); 2.31, 2.49 (2s, 4H, $2CH_2$); 3.38 (s, 2H, NH_2, D_2O exchangeable), 5.10 (s, 1H, -CH); 7.13-7.33 (m, 5H, 5 aromatic protons), 7.83 (s, 1H, NH, D_2O exchangeable), 8.54 (s, 1H, pyrimidine CH). Anal. Calcd. For Molecular Formula $C_{20}H_{21}N_5OS$: C, 63.30%; H, 5.58%; N, 18.46%; Found: C, 63.29%; H, 5.54%, N, 18.44%.

Reaction of aza-enamine (2) with malononitrile/3-methylpyrazol-5-one

General procedure

Carrying out these reactions under the same previous exp. Conditions, and refluxed for 26/30h. the reaction mixture was evaporated under reduced pressure. The residue **(11)** and **(12)** were crystallized from appropriate solvent.

2-{[(1E)-2,2-dicyanoethylidene]amino}-7,7-dimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide (11)

Yellow crystals, (EtOH), yield (77%); mp. 246 °C, Ms. m/z: 419 [M^+, 30%]. IR (KBr) ν cm^{-1}: 3450 (NH_2), 3181 (NH); 2956 (-CH_3); 1690 (C=O), 1662 (C=O), 1645 (C=N). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS): δ 1.01, 1.10 (2s, 6H, $2CH_3$); 2.21, 2.27 (2s, 4H, $2CH_2$); 2.57 (s, 3H, CH_3), 3.01 (d, 1H, CH), 3.47 (d, 1H, CH), 4.12 (s, 2H, NH_2, D_2O exchangable), 4.90 (s, 1H, -CH), 7.21-7.30 (m, 6H, 5 aromatic protons and 2NH), 12.20 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. For Molecular Formula $C_{23}H_{25}N_5O_3$: C, 65.85%; H, 6.01%; N, 16.70%; Found: C, 65.80%; H, 5.95%; N, 16.65%.

7,7-Dimethyl-2-[(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-4-ylmethylene)-amino]-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydro-quinoline-3-carboxylic acid amide (12)

Whit crystals, (EtOH), yield (35%); mp. 254 °C, Ms. m/z: 387 [M^+, 25%]. IR (KBr) ν cm^{-1}: 3435 (NH_2), 3160 (NH); 2956 (C-H, aliphatic); 2207 (CN), 1705 (C=O), 1662 (C=O). ^{1}H-NMR (270 MHz, $CDCl_3$, TMS): δ 0.99, 1.08 (2s, 6H, $2CH_3$); 2.12, 2.02 (2s, 4H, $2CH_2$); 2.55 (d, 1H, CH), 2.2 (d, 1H, CH), 3.39 (s, 2H, NH_2, D_2O exchangeable), 4.95 (s, 1H, -CH); 7.22-7.33 (m, 5H, 5 aromatic protons), 7.81 (s, 1H, pyridine NH). ^{13}C-NMR (270 MHz, DMSO-d_6, TMS): 17 (C7); 17.3 (CH_3, pyrazole); 26.7 (C4, pyridine); 26.8 ($2CH_3$); 46 (C4, pyrazole); 48.3 (C8); 54 (C6); 100.9 (C3); 108.2 (Ca?); 125.5, 128.4, 128.4, 129.2, 129.2, 137.7 (6C, phenyl); 145.4 (Cb?); 155.6 (C3, pyrazole); 163.7 (N=C); 168.3 (C=O, amide); 173 (C5, pyrazole); 197.6 (C5); Anal. Calcd. For Molecular Formula $C_{22}H_{21}N_5O_2$: C, 68.20%; H, 5.46%; N, 18.08%; Found: C, 68.17%; H, 5.40%; N, 18.02%.

BIOASSAY

Antitumor activity

Potential cytotoxicity of the compounds was tested using the method of Skehan et al[21]. Cells were plated in 96-multi well plates (104 cells/well) for 24 h. before treatment with the compounds allowing the attachment of cell to the wall of the plate. Different concentrations

of the tested compounds (0.0, 5.0, 12.5, 25 and 50 µg/ml) 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 °C and in an atmosphere containing 5% CO_2. After 48 h. cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between Surviving fraction and the drug concentration was plotted to get the Survival curve of each tumor cell line of the specified compound. The reference drug was used in the same concentrations as the test compounds.

CONCLUSION

Some new polyhydroquinoline derivatives were synthesized and investigated as antitumor agents against liver and breast cancer cells. The investigated compound **(5b)** exert the highest ant proliferative activity in human liver carcinoma and breast carcinoma cell lines HEPG2 and MCF7. The other tested compounds have a moderate activity as cytotoxic agents against cells under investigation.

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