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Research Article

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL QUINAZOLINE DERIVATIVES AS POTENTIAL ANTICANCER AGENT

AAF. Wasfy[1], OA. Fathalla[2] and AA. Salman[2*]

[1]Department of Chemistry, Faculty of Science, BenhaUniversity, Benha, Egypt.
[2]Department of Therapeutical Chemistry, National Research Center,Dokii, Egypt.

ABSTRACT

Novel derivatives of quinazolinehave been synthesized by reaction of 6-chloro-4-chloro-2-(pyridin-4-yl)quinazoline**4**with anthranilic acid. The newly synthesized compounds have been confirmed on the basis of spectral data (IR, ^{1}HNMR, mass) and physical data (MP, TLC and elemental analysis). Some ofthe synthesized compounds were screened for human liver cell line (HepG2) activity. Almost all of them demonstrated good activity anticancer.

Keywords: Quinazoline, Synthesis, Anticancer.

INTRODUCTION

Quinazoline derivatives are reported to be physiologically and pharmacologically active.[1]They also exhibit a wide range of activitiessuch as anticonvulsant, anti-inflammatory, antifungal, antimalarial and sedative.[2-6]some of these compounds are identified as drugs[7] used as diuretics, vasodilators and antihypertensive agents.

Experimental

Chemicals and solvents used were of reagent grade and used without further purification.The purity of the synthesized compounds was determined by melting point using opencapillary method and is uncorrected. IR (infrared) was performed usingJasco FT/IR-6100 using KBr discs. The compounds **1-11**were identified by ^{1}HNMR(proton nuclear magneticresonance) usingJeol 270 MHz and jeolsx 500MHz spectrometers using TMS as internal standard, Mass spectra were acquired with a Joel JMS-AX 500. All reactions were followed and checked by TLC (aluminum-backed plates) with Ethyl acetate-Petrolum ether 2:8 as mobile phase. For detection the plates were sprayed with iodine.

MATERIALS AND METHODS

Synthesis of compounds 1-3 (Scheme 1)

5-Chloro-2-(isonicotinamido)benzoic acid 1

A mixture of 5-chloroanthranilic acid (0.01 mol) and isonicotinyl chloride (0.01 mol) in dry pyridine (30 mL) was stirred at room temperature for 12h. The reaction mixture was poured onto ice/water then, acidified with diluted HCl, the formed precipitated solid was then filtered off and recrystallized from acetic acid to give compound**1**.

6-Chloro-2-(pyridin-4-yl)-4*H*-benzo[d][1,3]oxazin-4-one 2

A mixture of compound **1** (0.01 mol) and acetic anhydride (5 mL) was heated together upon fusion at 150 ^{o}C on sand bath for 2h. After cooling, the crude mass was recrystallized from ethanol to give dark brown crystals of compound **2**.

6-Chloro-2-(pyridin-4-yl)quinazolin-4(3*H*)-one 3

Procedure A

A mixture compound **1** (0.01 mol), ammonium acetate (0.01 mol), ammonium hydroxide (2 mL) and 10% sodium hydroxide (5 mL) in pyridine (15 mL), was heated under reflux for 2h. Then left to cool.The reaction mixture was

then triturated with cold water (50 mL) and neutralized with 1N HCl (5 mL) the resulting precipitated solid was collected by filtration, washed with water, dried and recrystallized from ethanol to give compound **3.**

Procedure B
A mixture of compound **2** (0.01 mol) and ammonium acetate (0.01 mol) in ethanol (50 mL), was refluxed for 5h. The mixture then cooled and the separated solid was filtered off and recrystallized to give compound **3**.

Synthesis of compounds 4-7 (Scheme 2)
6-chloro-4-chloro-2-(pyridin-4-yl)quinazoline 4
A mixture of compound**3** (0.01 mol) and phosphorus pentachloride (0.015 mol) in phosphorus oxychloride (20 mL) was heated on a water bath for 8 h. and the reaction mixture poured gradually onto crashed ice. The separated solid was filtered off, dried then recrystallized from acetic acid to give compound **4**.

6-chloro-N-(aryl)-2-(pyridin-4-yl)quinazolin-4-amine 5a,b
General procedure
A mixture of compound **4** (0.01 mol) and aromatic amines namely, 4-chloro-aniline and/or 4-aminoantipyrine (0.01 mol) and few drops of piperidine in methanol (25 mL) was heated under reflux for 10h. The precipitated solid was filtered off, dried then recrystallized from methanol to give compounds **5a,b** respectively.

6-chloro-4-hydrazinyl-2-(pyridin-4-yl)quinazoline 6
A mixture of compound**4** (0.01 mol) and hydrazine hydrate (0.015 mol) in methanol (15 mL) was stirred for 8h. The Solid precipitate was filtered off, dried under vacuum then recrystallized from acetic acid to give compound **6**.

9-chloro-3-methyl-5-(pyridin-4-yl)-[1,2,4]triazolo[4,3-*c*]quinazoline 7
A mixture of**4** (0.01 mol) and acetyl hydrazine (0.01 mol) in n-butanol (30 mL) was heated under reflux for 8h. The obtained solid after cooling was dried and recrystallized from dimethylformamide to give compound **7**.

Synthesis of compounds 8-11 (Scheme 3)
2-Chloro-6-pyridin-4-yl-quinazolino[4,3-b]quinazolin-8-one 8
A mixture of compound **4** (0.01 mol) and anthranilic acid (0.01 mol) in n-butanol (30 mL) was heated under reflux for 10h. The obtained product which formed was collected and recrystallized from dioxane to give compound **8**.

9-chloro-5-(pyridin-4-yl)tetrazolo[1,5-*c*]quinazoline 9
A mixture of compound**4** (0.01 mol) and sodium azide (0.01 mol) in ethanol (30 mL) was heated under reflux for 5h. The product obtained after cooling was dried recrystallized from dioxane to give compound **9**.

9-chloro-5-(pyridin-4-yl)imidazo[1,2-*c*]quinazolin-3(2*H*)-one 10
A mixture of compound**4** (0.01 mol) and glycine (0.01 mol) in acetic acid (10 mL) was heated under refluxed for 8h. The solid product formed upon pouring onto ice/water was collected by filtration and recrystallized from methanol to give orange crystals of compound **10**.

1-(4-(6-chloro-2-(pyridin-4-yl)quinazolin-4-ylamino)phenyl)ethanone 11
A mixture of compound **4** (0.01 mol) and *p*-aminoacetophenone (0.01 mol) in pyridine (30 mL) was refluxed for 6h and the reaction mixture poured gradually on water and then neutralized till acidification. The precipitate was filtered off, dried then recrystallized from methanol to give yellow crystals of compound **11**.

Biological Activity
Anti-cancer activity
In view of the biological activity possessed byquinazoline, some of the newly synthesized compounds (**3, 4, 8, 9**and**11**) were evaluated for anticancer activities using the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to test them in vitro cytotoxicity against human hepatocellular liver carcinoma (HepG2).
The tested compounds were initially screened at the single concentration of 0.06µM to test their in vitro cytotoxicity against human hepatocellular liver carcinoma (HepG2). The standard drug used in the anticancer screening is Doxorubicin (0.009µM).

RESULTS AND DISCUSSION
Synthesis of compounds 1-3 (Scheme 1)
The starting material 5-Chloro-2-(isonicotinamido)benzoic acid **1** was prepared bythe reaction of 5-chloro anthranilicacid with isonicotonyl chloride in dry pyridine. Thus IR spectrum of compound**1**indicated absorption bands at υ = 3498 (OH), 3125 (NH), 3030 (CH-aromatic), 1680 (CO of COOH), 1652 (CO of CONH), 1615 (C=N).

Compound **1** was boiled with acetic anhydride to give 6-Chloro-2-(pyridin-4-yl)-4*H*-benzo[d][1,3]oxazin-4-one **2.**The IR spectrum of compound**2** indicated absorption bands at υ = 3035 (CH-aromatic), 1675 (CO), 1626 (C=N)**.** Interaction of 5-chloro-2-(isonicotinamido)benzoic acid **1** with ammonium acetate in the presence of ammonium hydroxide in oil bath afforded easily separated and highly yield product 6-chloro-2-(pyridin-4-yl)quinazolin-4(3*H*)-one**3** Thus IR spectrum of compound **3** exhibited absorption bands at υ = 3249 (NH), 3074 (CH-aromatic), 1688 (CO), 1620 (C=N).On the other hand, when compound **2** was reacted with ammonium acetate gave compound **3**.

Synthesis of compounds 4-7 (Scheme 2)

The chlorination of compound **3**with phosphorusoxy chloride in the presence of phosphoruspenta chloride afford the 6-chloro-4-chloro-2-(pyridin-4-yl)quinazoline**4**Thus IR spectrum of compound**4** exhibited absorption bands at υ = 3020 (CH-aromatic),1620 (C=N) **.** Refluxing of compound **4** with aromatic amines namely *p*-chloroaniline and/or aminoantipyrine, afforded 6-chloro-*N*-(4-chlorophenyl)-2-(pyridin-4-yl)quinazolin-4-amine **5a.** Thus IR spectrum of compound **5a** exhibited absorption bands at υ = 3178 (NH), 3070 (CH-aromatic), 1616 (C=N)and 4-(6-chloro-2-(pyridin-4-yl)quinazolin-4-ylamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one **(5b)**Thus IR spectrum of compound**5b** exhibited absorption bands at υ = 3179 (NH), 3076 (CH-aromatic), 1686 (CO), 1602 (C=N). Hydrazonolysis of compound**4** gave the corresponding 6-chloro-4-hydrazinyl-2-(pyridin-4-yl)quinazoline**6.**Thus IR spectrum of compound **6** exhibited absorption bands at υ = 3328,3213 (NH_2), 3125 (NH), 3001 (CH-aromatic), 1620 (C=N)**.** Refluxing of compound **4** with acetohydrazide gave the corresponding 9-chloro-3-methyl-5-(pyridin-4-yl)-[1,2,4] triazolo[4,3-*c*]quinazoline**7**. Thus IR spectrum of compound **7** exhibited absorption bands at υ = 3021 (CH-aromatic), 2984 (CH-aliphatic), 1620 (C=N).

Synthesis of compounds 8-11 (Scheme 3)

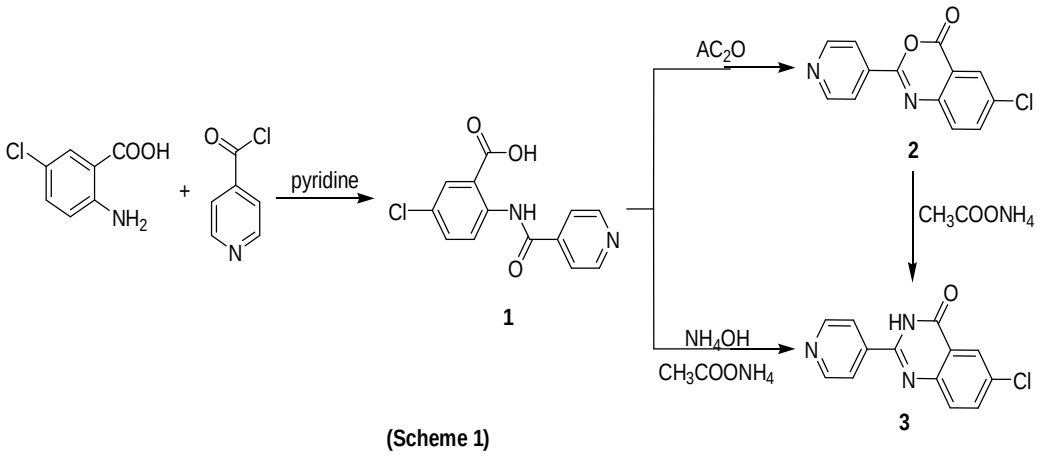
On the other hand, when compound **4** reacted with anthranilic acid in n-butanol gave 2-Chloro-6-pyridin-4-yl-qunazolino[4,3-b] quinazolin-8-one **8**Thus IR spectrum of compound**8** exhibited absorption bands at υ = 3012 (CH-aromatic), 1700 (CO), 1619 (C=N) .Also by refluxing of compound **4** with Sodium azide afforded 9-chloro-5-(pyridin-4-yl)tetrazolo[1,5-*c*]quinazoline **9**Thus IR spectrum of compound**9** exhibited absorption bands at υ = 3100 (CH-aromatic),1620 (C=N) **.** Cyclocondensation of compound **4** with glycine afforded 9-chloro-5-(pyridin-4-yl)imidazo[1,2-*c*]quinazolin-3(2*H*)-one **10**Thus IR spectrum of compound **10** exhibited absorption bands at υ = 3080 (CH-aromatic), 2921 (CH-aliphatic), 1687 (CO), 1620 (C=N) **.** Interaction of compound**4** with *p*-aminoacetophenone gave 1-(4-(6-chloro-2-(pyridin-4-yl)quinazolin-4-ylamino) phenyl) ethanone**11**Thus IR spectrum of compound**11** exhibited absorption bands at υ = 3225 (NH), 3099 (CH-aromatic), 2937 (CH-aliphatic), 1680 (CO), 1627 (C=N)**.**

All the compounds were synthesized in reasonably good yields and high purity. The structures ofnewly synthesized compounds wereelucidated by spectral data viz., IR, ^{1}HNMR,and Mass and characterized by physical data viz., melting point, TLC, elemental analysis. Some of thecompounds showed significant anti-cancer (**3, 4, 8, 9** and **11)**

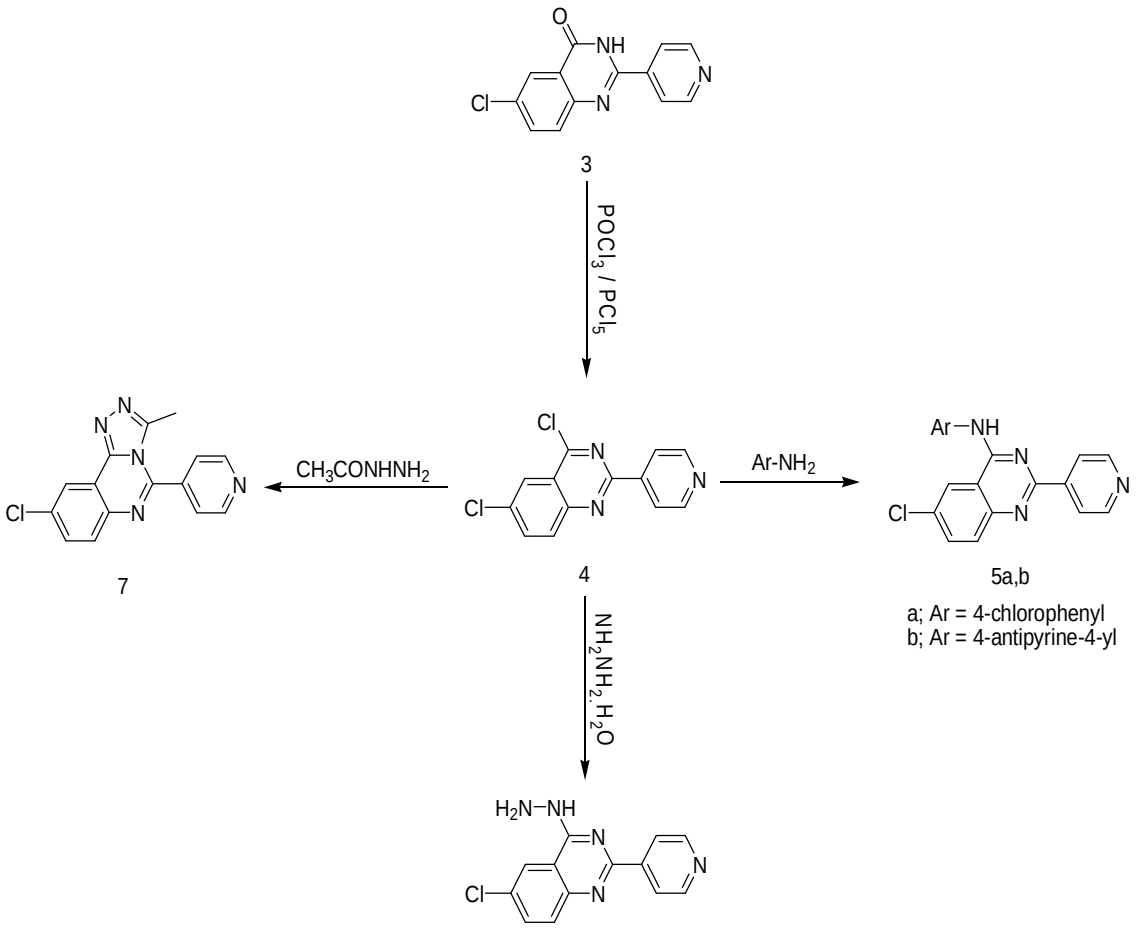
CONCLUSION

The structures of the newly synthesized compounds **1-11**were confirmed by spectraldata viz. IR, ^{1}H NMR,Mass spectra and elementary analysis. Some of the synthesized final compounds **3,4,8,9** and **11** were screened for Anti-cancer activity against human hepatocellular liver carcinoma (HepG2) using Doxorubicin as standard reference.

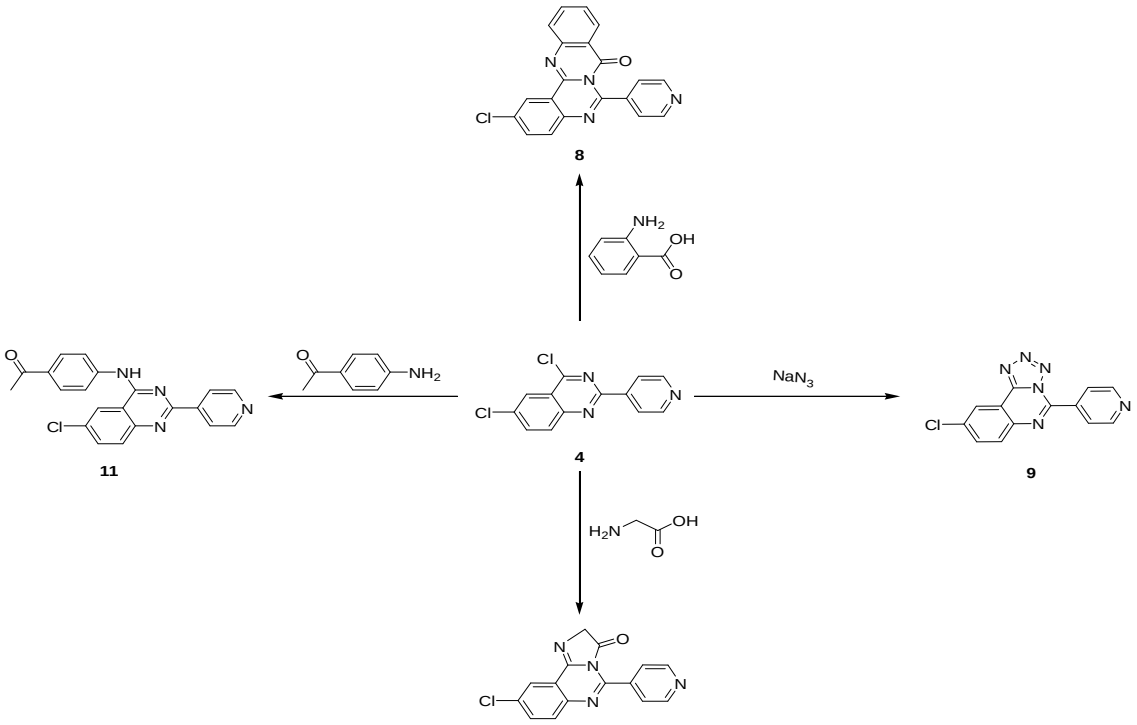
Some of thequinazoline derivatives have shown significant activity and with these encouraging results, all the synthesized compounds can be further explored for structural modification and detailed microbiological investigations to arrive at possibly newer potent moieties with better therapeutic activity.



(Scheme 1)



(Scheme 2)



(Scheme 3)

Table 1: Physical data for Synthesized Compounds

Compound	M.P ˚C	%Yield
1	205-209	82%
2	195-197	82%
3	185-187	78%
4	130-135	82%
5a	270-272	80%
5b	179-181	75%
6	241-244	73%
7	295-297	60%
8	150-152	65%
9	>300	66%
10	260	64%
11	120-124	65%

Table 2: TLC data for synthesized Compounds

Compound	Mobile Phase(Ratio)	R^f
1	Ethylacetate:petrol um ether(2:8)	0.34
2	Ethylacetate:petrol um ether(2:8)	0.55
3	Ethylacetate:petrol um ether(2:8)	0.22
4	Ethylacetate:petrol um ether(2:8)	0.54
5a	Ethylacetate:petrol um ether(2:8)	0.56
5b	Ethylacetate:petrol um ether(2:8)	0.29
6	Ethylacetate:petrol um ether(2:8)	0.73
7	Ethylacetate:petrol um ether(2:8)	0.13
8	Ethylacetate:petrol um ether(2:8)	0.78
9	Ethylacetate:petrol um ether(2:8)	0.33
10	Ethylacetate:petrol um ether(2:8)	0.54
11	Ethylacetate:petrol um ether(2:8)	0.76

Table 3: Spectral (IR and Mass) data for synthesized compounds

Compound	IR(KBr,cm^{-1})	Mass
1	υ = 3498 (OH), 3125 (NH), 3030 (CH-aromatic), 1680 (CO of COOH), 1652 (CO of CONH), 1615 (C=N).	276.5
2	υ = 3035 (CH-aromatic), 1675 (CO), 1626 (C=N).	258.5
3	3249(NH),3074(Ar ,C-H),1688(CO),1620(C=N)	257.5
4	3020(Ar , C-H) ,1620(C=N)	276
5a	υ = 3178 (NH), 3070 (CH-aromatic), 1616 (C=N).	368
5b	υ = 3179 (NH), 3076 (CH-aromatic), 1686 (CO), 1602 (C=N).	443
6	υ = 3328, 3213 (NH_2), 3125 (NH), 3001 (CH-aromatic), 1620 (C=N).	271
7	υ = 3021 (CH-aromatic), 2984 (CH-aliphatic), 1620 (C=N).	295
8	3012(Ar , C-H) ,1700(CO),1619(C=N)	358.5
9	3100(Ar , C-H),1620(C=N)	282
10	υ = 3080 (CH-aromatic), 2921 (CH-aliphatic), 1687 (CO), 1620 (C=N).	296
11	3225(NH),3099(Ar , C-H),2937(Aliphatic,C-H),1680(C=O),1627(C=N)	374

Table 4: Spectral (^{1}HNMRand Elemental analysis) datafor synthesized compounds

Compound	^{1}HNMR (MEOD,ppm)	Elemental analysis		
		Carbon	Hydrogen	Nitrogen
1	6.7-7.3 [7H, Ar-H], 9.7 [1H, NH, D_2O exchangeable], 12.1 [1H, OH, D_2O exchangeable]	48.60	2.83	8.73
2	6.7-7.8 [7H, Ar-H]	51.49	2.31	9.22
3	7.2-8.2[7H,Ar],10[1H,NH,D_2O exchangeable]	51.66	2.65	13.89
4	6.7-8.2 [7H, Ar].	48.69	2.18	13.09
5a	7.2,7.6 [4H, AB-ArH, *J*=8.1 *Hz*], 7.9-8.6 [7H, Ar-H], 10.1 [br, 1H, NH, D_2O exchangeable]	55.41	2.92	13.59
5b	2.4[3H, C-CH_3], 3.7 [3H, N-CH_3], 6.7-7.7 [12H, Ar-H], 9.7[br, 1H, NH, D_2O exchangeable].	59.13	3.91	17.22
6	5.3[br, 2H, NH_2, D_2O exchangeable], 7.8-8.8 [7H, Ar-H], 9.5[1H, NH, D_2O exchangeable]	49.37	3.17	22.13
7	2.5[3H, CH_3], 7.2-7.9[7H, Ar-H].	52.94	2.94	20.57
8	7.5-8.6 [11H, Ar-H].	59.55	2.73	13.87
9	7.4-8.1 [7H, Ar-H].	47.71	2.14	25.67
10	4.5 [2H, CH_2], 7.6-8.5 [7H, Ar-H].	52.79	2.64	16.40
11	2.6 [3H, CH_3], 6.8-7.3 [7H, Ar-H] 7.7,8.3 [4H, AB-ArH, *J*=8.6 *Hz*] 10.2 [1H, NH, D_2O exchangeable].	60.14	3.59	13.34

Table 5: Anticancer activity of some synthesized compounds(3,4,8,9 and 11)

Compound	Cytotoxocity(IC_{50} in µg)
	HepG2
3	32.41+/ - 6.37
4	52.01 +/ -8.62
8	1.01 +/ - 0.52
9	12.01 +/- 0.10
11	16.32 +/- 2.27
Doxorubicin	0.009 µM

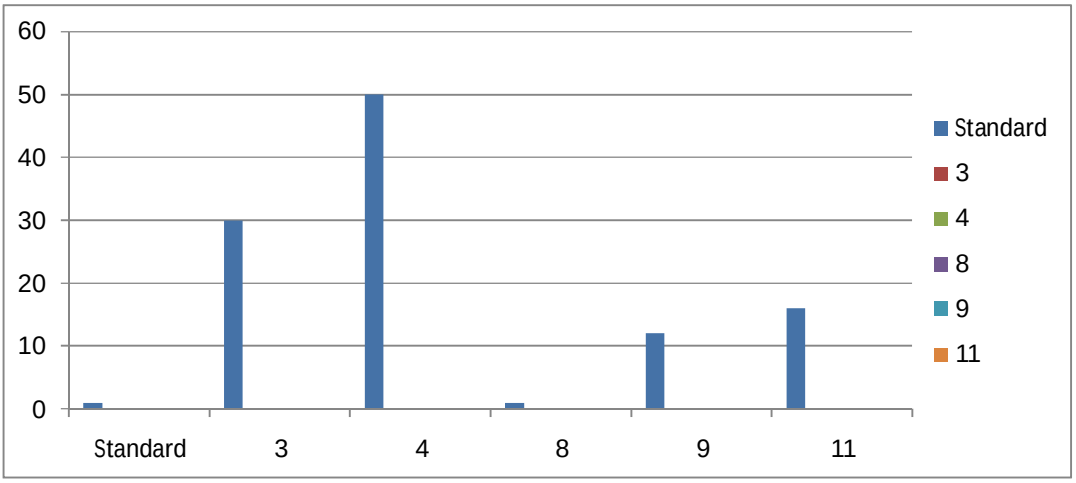


Fig. 1: Graphical representation of anticancer activity of compounds (3, 4, 8, 9 and 11)

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