Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(14):100-110
(http://derpharmachemica.com/archive.html)

Synthesis and molecular modeling of new quinoline derivatives as antitumor agents

Lamiaa Osama*, Laila A. Abou-Zeid, Magda N. A. Nasr and Atif S. Tantawy

Department of Pharmaceutical organic Chemistry, Faculty of Pharmacy, Mansoura University 35516, Egypt

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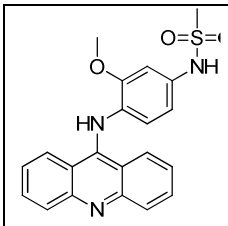
ABSTRACT

A new series of quinoline containing compoundswasdesigned,synthesized and evaluated biologically for their invitro antitumor activity. Compounds ***7, 8,11, 12,17*** *and* ***18****showed the best cytotoxic activity against five tumors cell lines (HePG-2, HCT-116, MCF-7, PC3 and Hela) with* IC_{50} *range between 5.6-19.2 µg/ml. Molecular modeling studies have been performed for the investigated compounds to evaluate their recognition profiles at the VGFR tyrosine kinase binding-pocket.*

Keywords: Quinoline; VGFR inhibition;molecular modeling and antitumor activity.

INTRODUCTION

Quinoline is asignificant nucleus that found in many natural and synthetic products with various pharmacological activities. The prominent ones have antimalarial [1], antibacterial [2],antifungal [3], antiinflamatory [4], and anticanceractivities [5].



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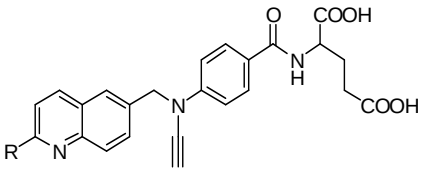
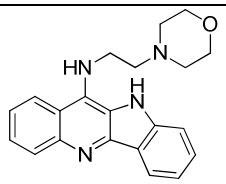


2-(4-[((2-substituted quinolin-6-yl)methyl)(ethynyl)amino)ben
pentanedioic acid)

II



N-(2-morpholinoethyl)-10*H*-indolo[3
quinolin-11-amine

III

Figure 1: Quinoline containing antitumor agents

Cancer is one of the major health problems in developing as well as undeveloped countries causing death worldwide [6]. From this point, many researches were accomplished to afford potential safe promising anticancer leads. In the current study, the quinoline ring was selected as an essential core due to its well-known antiproliferative activity

through inhibition of different enzymes including topoisomerase (I) [7,8],thymidylate synthase(II) [9], telomerase (III) [10,11], and different protein kinases [12,13].**(Figure 1)**

Among different protein kinase inhibitors, VEGF–TKIs (vascular endothelial growth factor receptor- tyrosine kinase inhibitors) are valuable kind of recently targeted cancer therapy [14,15]. The function of (VEGF) in cancer is not limited to angiogenesis and vascular permeability. VEGF-mediated signaling occurs in tumour cells and this signaling contributes to key aspects of tumorigenesis and tumour initiation, so its inhibition would be a promising strategy for controlling different tumors [16,17]. Varied quinoline-containing drugs such as Tivozinib [18],Ki 8751 [19]and Lenvatinib [20] were recently approved for treatment of various solid tumors as VGFR–TKIs.

Inspired by the previous rationale, new series of 2-aryl-4,6-disubstituted quinolines have been synthesized, the derived 4-substituted quinoline pharmacophores are structurally related to Tivozinib, Ki 8751 and Lenvatinib by insertion of lipophilic -substituted phenyl at position 2 of quinoline ring. In addition; substituting amide or methoxy moiety by lipophilic halogen at position 6 that improves the lipophilicity, therefore enhances the activity towards cancerous cells.

Moreover, replacement of the urea linkage by amide (compounds **7, 8**, **11, 12, 13, 14, 15** and **16**) or carbonyl (compounds **9** and **10**) functions was performed to investigate the significance of the hydrophilic spacer between the quinoline core and the peripheral ring substitutions. Fluorinated or chlorinated phenyl group were replaced with different rings to get the appropriate assumption about the appropriate necessities of hydrophilic region. The terminal Tivozanib' isoxazole ring is replaced by alternative heterocyclic groups such as oxadiazole(compounds **17** and **18**) or pyrazole (compounds **9** and **10**).**(Figure 2)**

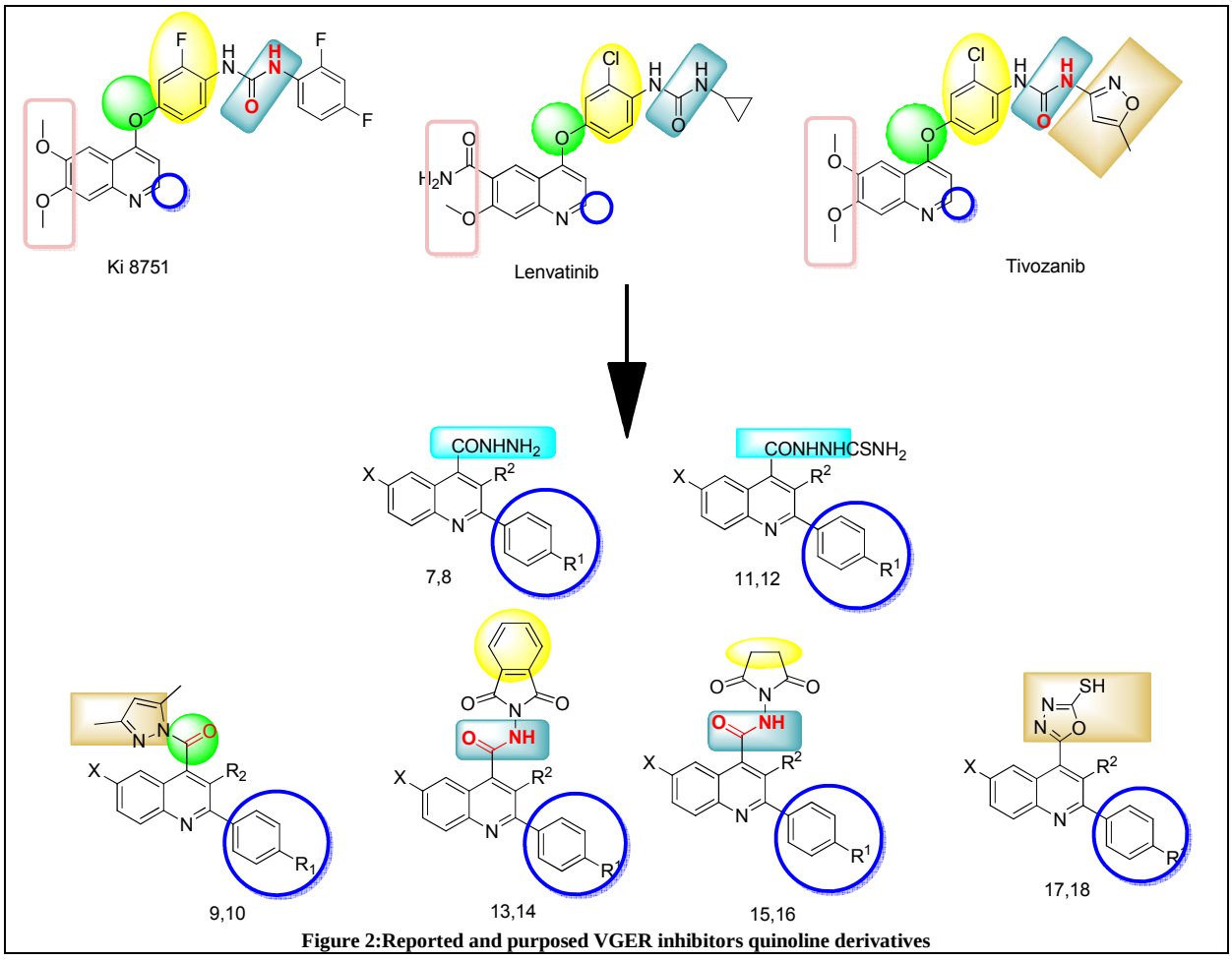


Figure 2:Reported and purposed VGER inhibitors quinoline derivatives

MATERIALS AND METHODS

Melting points (ºC) were recorded using *Fisher-John* melting point apparatus and are uncorrected. Microanalyses were performed at the microanalytical unit, Cairo University. IR spectra were recorded on Mattson 5000 FT-IR spectrometer (υ in cm-1) using KBr disk. The ^{1}H - NMR and ^{13}C -NMR spectra were recorded on Bruker Ac 400 FT NMR spectrometer (400 MHz) in DMSO-d_6,Cairo, Egypt**.** The chemical shifts in ppm are expressed in δ units using tetramethylsilane (TMS) as internal standard. MS analyses were performed on JOEL JMS-600H spectrometer in Cairo University. Reaction times were determined using TLC technique on Silica gel plates 60 F245E. Merk, and the spots were visualized by U.V. (366 nm).Compounds**5-substituted indoline-2,3-diones (1, 2)** were synthesized according to the reported method**.**[14]

General procedure for preparation of compounds3 and 4

A mixture of the appropriate 4-substituted acetophenone or propiophenone(5mmol), prepared 5-substituted isatin1 or 2 (5mmol) and potassium hydroxide (1.28 g, 23mmol) in 50% aqueous ethanol (20 mL) was refluxed for 23 h. After cooling to the room temperature, the reaction mixture was diluted with 30% aqueous ethanol (20mL) and neutralized with 50% acetic acid solution. The formed precipitated solid was filtered, dried and recrystallized from ethanol.

6-Bromo-2-(4-methylphenyl)quinoline-4-carboxylic acid (3)

Yellow crystals, yield (74%), m.p.268□C. IR (KBr, cm^{-1}): 3424 very broad (OH), 1714 (C=O).^{1}H-NMR (DMSO-d_6,δ ppm): 2.38 (s, 3H, CH_3), Ar-H : δ 7.35 (d, *J*=7 Hz, 2H, 2-phenyl ring), 7.89 (d, *J*=8 Hz , 1H, C-7 quinoline), 8.02 (d, *J*=8 Hz, 1H, C-8 quinoline), 8.16 (d, *J*= 7 Hz, 2H, 2-phenyl ring), 8.40 (s, 1H, C-3 quinoline), 9.00(s, 1H, C-5 quinoline),, 10.00 (1H, COOH, D_2O exchangeable). ^{13}C-NMR(DMSO-d_6): δ 21.3, 119.4, 124.8, 125.7, 128.1, 129.3, 131.0, 133.0, 135.7, 138.4, 140.1, 141.7, 147.4, 156.7, 168.6. MS: (□ /□%) 342[M^+], 344[M^++2]. Anal.calcd for $C_{17}H_{12}BrNO_2$: C, 59.67; H, 3.53; N, 4.09. Found: C, 59.42; H, 3.45; N, 4.02.

2-(4-Bromophenyl)-6-chloro-3-methylquinoline-4-carboxylic acid(4)

Pale yellow crystals, yield (71%), m.p.>300□C. IR (KBr,cm^{-1}) 3447 very broad (OH), 1709 (C=O).^{1}H-NMR (DMSO-d_6,δ ppm): 2.5 (s, 3H, CH_3),Ar-H : δ 7.60 (d, *J*= 8 Hz, 2H, 2-phenyl ring), 7.73 (d, *J*= 8 Hz, 2H, 2- phenyl ring), 7.80 (m, 2H, C-7,8 quinoline), 8.10 (d, 1H, C-5 quinoline),, 10.20 (1H, COOH, D_2O exchangeable). MS: (□ /□%) 376.6[M^+], 378.9[M^++2]. Anal. calcd for $C_{17}H_{11}BrClNO_2$: C, 54.21; H, 2.94; N, 3.72. Found: C, 54.11; H, 2.77; N, 3.64.

General procedure for preparation of compounds 7and 8

A mixture of 6-substituted-2-arylquinoline-4-carboxylic acids (10 mmol), thionyl chloride (2.4 g, 20 mmol), and few drops of DMF in methylene chloride (50 mL) was refluxed overnight. The reaction mixture was evaporated under vacuum, the produced acid chlorides **(5**or**6)** were dissolved in absolute ethanol (10 mL) without further purification, hydrazine hydrate (99%; 3 mL) was added, refluxed for 8 h. After cooling, the reaction mixture was evaporated under vacuum and the crude product was purified by recrystallization from ethanol.

6-Bromo-2-(4-methylphenyl)quinoline-4-carbohydrazide (7)

Yellowish white crystals, yield (75%),m.p. 270□C. IR (KBr,cm^{-1}) 1613 (C=O amide), 3446 (NH str.).^{1}H-NMR (DMSO-d_6,δ ppm): 2.5 (s, 3H, CH_3), 3.4 (s, 2H, NH_2, D_2O exchangeable), Ar-H: δ 7.3-7.4 (m, 3H), 7.86 (m, 1H), 7.99 (d, 1H), 8.1 (d, 2H), 8.33 (s, 1H),, 9.1 (s, 1H, NH, D_2O exchangeable).MS: (□ /□%) 356[M^+]. Anal.calcd for $C_{17}H_{14}BrN_3O$: C, 57.32; H, 3.96; N, 11.80. Found: C, 57.37; H, 3.99; N, 11.78.

2-(4-Bromophenyl)-6-chloro-3-methylquinoline-4-carbohydrazide (8)

White crystals, Yield (73%), m.p. 262□C. IR (KBr,cm^{-1}):1611 (C=O amide), 3316 (NH str.).^{1}H-NMR (DMSO-d_6,δ ppm): 2.51 (s, 3H, CH_3), 4.7 (s, 2H, NH_2, D_2O exchangeable), Ar-H: δ 7.5 (d, 2H), 7.7 (d, 2H), 7.75 (d, 1H), 7.84 (d, 1H), 8.07 (d, 1H),, 9.7 (s,1H, NH, D2O exchangeable). MS: (□ /□%) 390.9[M^+], 392.6[M^++2]. Anal.calcd for $C_{17}H_{13}BrClN_3O$: C, 52.27; H, 3.35; N, 10.76. Found: C, 52.11; H, 3.30; N, 10.72.

General procedure for synthesis of compounds 9 and 10
A mixture of 7 or 8 (10 mmol) and acetylacetone (1 g; 10 mmol) in glacial acetic acid (15 mL) was refluxed for 24h. After cooling, the reaction mixture was poured onto ice-water, filtered, washed with water, dried, and recrystallized from ethanol.

[6-Bromo-2-(4-methylphenyl)quinolin-4-yl](3,5-dimethyl-1*H*-pyrazol-1-yl)methanone (9)Yellow crystals, yield (51%), m.p. 257□C.IR (KBr, cm^{-1}): 1654 (C=O). ^{1}H-NMR, (DMSO-d_6,δ ppm): 1.45 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.3 (s, 3H, CH_3), 4.5 (s, 1H, =C**H**),), Ar-H : δ 7.4 (d, 2H), 7.94 (m, 1H), 8.05 (m, 1H), 8.19 (m, 2H), 8.4(d, 1H), 8.8 (s,1H).MS:(θ /∀%) 420[M^+], 422[M^++2]. Anal. calcd for $C_{22}H_{18}BrN_3O$: C, 62.87; H, 4.32; N, 10.00. Found: C, 62.77; H, 4.29; N, 10.08.

[2-(4-Bromophenyl)-6-chloro-3-methylquinolin-4-yl](3,5-dimethyl-1*H*-pyrazol-1-yl)methanone (10)
White crystals, yield (56%), m.p.177□C.IR (KBr, cm^{-1}): 1651 (C=O), 1585 (C=N).^{1}H-NMR (DMSO-d_6,δ ppm): 2.03 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.75 (s, 3H, CH_3), 6.39 (s, 1H, =C**H**), Ar-H : δ 7.64-7.67 (m, 3H), 7.74 (m, 2H), 7.82 (d, 1H), 8.12 (d, 1H). MS: (□ /□%) 454.7[M^+]. Anal. calcd for $C_{22}H_{17}BrClN_3O$: C, 58.11; H, 3.77; N, 9.24. Found: C, 58.18; H, 3.80; N, 9.28.

General procedure for synthesis of compounds 11 and 12
The appropriate acid hydrazide (1.5 mmol) was mixed with dilHCl(15 mL, 10%) and ethanol (5 mL), and then potassium thiocyanate (0.145g; 1.5 mmol), the mixture was heated under reflux for 48h. After cooling, the precipitated solid product was filtered, washed with water, driedand recrystallized from DMF/ethanol mix.

[6-Bromo-2-(4-methylphenyl)quinoline-4-carbonyl]hydrazinecarbothioamide (11)
Yellow crystals, yield (44%),m.p.>300□C. IR (KBr, cm^{-1}): 1624 (C=O), 3447 (NH).^{1}H-NMR (DMSO-d_6,δ ppm): 2.46 (s, 3H, CH_3), 5.4 (s, 2H, NH_2, D_2O exchangeable), Ar-H : δ 7.4 (d, 2H), 7.92-7.97 (m,2H), 8.07 (d, 1H), 8.15-8.2 (m, 1H), 8.4 (s, 1H), 8.56 (s, 1H),, 9.49 (s, 1H, NH, D_2O exchangeable), 10.8 (s, 1H, NH, D_2O exchangeable).MS: (□ /□%) 415[M^+], 417[M^++2]. Anal. calcd for $C_{18}H_{15}BrN_4OS$: C, 52.06; H, 3.64; N, 13.49. Found: C, 52.02; H, 3.71; N, 13.35.

[2-(4-Bromophenyl)-6-chloro-3-methylquinoline-4-carbonyl]hydrazinecarbothioamide (12)
Yellow crystals, yield (46%), m.p.242□C. IR (KBr, cm^{-1}):1668(C=O), 3422(NH).^{1}H-NMR (DMSO-d_6,δ ppm): 2.48 (s, 3H, CH_3), 4.5 (s, 2H, NH_2, D_2O exchangeable), Ar-H : δ 7.46(d, 2H), 7.56-7.6 (m, 2H), 7.78 (d, 1H), 7.94 (m, 1H), 8.1(s, 1H),,9.8 (s, 1H, NH, D_2O exchangeable), 10.68 (s, 1H, NH, D_2O exchangeable). MS: (□ /□%) 449.9[M^+]. Anal. calcd for $C_{18}H_{14}BrClN_4OS$: C, 48.07; H, 3.14; N, 12.46. Found: C, 48.03; H, 3.09; N, 12.40.

General procedure for synthesis of compounds 13 and 14
A mixture of equimolar of the acid hydrazide (7 or 8) and phthalic anhydride (1.48 g; 10 mmol) in glacial acetic acid (15 mL) as a solvent, refluxed for 24 h. After cooling to room temperature the reaction mixture was poured into a suitable amount of crushed ice with stirring, the formed precipitate was filtered, washed with water, and recrystallized from ethanol.

6-Bromo-*N*-(1,3-dihydro-1,3-dioxoisoindol-2-yl)-2-(4-methylphenyl)quinoline-4-carboxamide (13)
Pale yellow crystals, yield (69%), m.p.286□C. IR (KBr, cm^{-1}): 3424 (NH), 1742, 1687 (two C=O, cyclic amide), 1610 (C=O, **CO**NH).^{1}H-NMR (DMSO-d_6,δ ppm): 2.42 (s, 3H, CH_3), Ar-H : δ 7.43 (d, 2H), 8.02-8.14 (m, 6H), 8.28 (d,2H), 8.36 (s, 1H), 8.55 (s, 1H),, 11.8 (s, 1H, NH, D_2O exchangeable).^{13}C-NMR (DMSO-d_6): δ 21.0, 118.1, 120.8, 121.3, 124.5, 127.4, 127.8, 128.0, 129.9, 130.1, 130.8, 132.2, 134.0,138.6, 140.6, 146.9, 156.6, 165.5, 169.1. MS: (□/□%) 486[M^+], 487[M^++1]. Anal. calcd for $C_{25}H_{16}BrN_3O_3$: C, 61.74; H, 3.32; N, 8.64. Found: C, 61.81; H, 3.22; N, 8.59.

2-(4-Bromophenyl)-6-chloro-*N*-(1,3-dihydro-1,3-dioxoisoindol-2-yl)-3-methylquinoline-4-carboxamide(14)

White crystals, yield(60%), m.p.> 300°C. IR (KBr, cm^{-1}): 3421(NH), 1733, 1675 (two C=O).^{1}H-NMR (DMSO-d_6,δ ppm): 2.5 (s, 3H, CH_3), Ar-H : δ 7.6 (d, 2H), 7.74-7.77 (d, 2H), 7.82-7.85 (d, 1H), 7.9-8.1 (m, 4H), 8.12 (m, 1H), 8.2 (s, 1H),, 11.2 (s, 1H, NH, D_2O exchangeable).MS:(□/□%) 520.8[M^+]. Anal. calcd for $C_{25}H_{15}BrClN_3O_3$: C, 57.66; H, 2.90; N, 8.07. Found: C, 57.53; H, 2.98; N, 8.12.

General procedure for synthesis of compounds 15 and 16

An appropriate acid hydrazide (7 or 8) and succinic anhydride (0.5 g; 5mmol) were mixed in glacial acetic acid (10 mL) as a solvent, refluxed for 24 h. After cooling to room temperature the reaction mixture was poured into a suitable amount of crushed ice, the formed precipitate was filtered, washed with water and recrystallized from ethanol.

6-Bromo-*N*-(2,5-dihydro-2,5-dioxopyrrol-1-yl)-2-(4-methylphenyl)quinoline-4-carboxamide(15)

White crystals, yield (75%), m.p. 218°C. IR (KBr, cm^{-1}): 3424(NH), 1774, 1732 (two C=O cyclic amide), 1657 (**CO**NH). ^{1}H-NMR (DMSO-d_6,δ ppm): 2.01(s, 3H,CH_3), 2.9 (s, 4H, $2CH_2$), Ar-H: δ 7.9 (m, 2H), 8.1 (m, 2H), 8.1-8.2 (m, 2H), 8.52 (s, 1H), 8.59 (s, 1H),, 10.1 (s, 1H, NH, D_2O exchangeable). MS: (□/□%) 439 [M^++1], 440 [M^++2]. Anal. calcd for $C_{21}H_{16}BrN_3O_3$: C, 57.55 ; H, 3.68 ; N, 9.59. Found: C, 57.45; H, 3.72; N, 9.47.

2-(4-Bromophenyl)-6-chloro-*N*-(2,5-dihydro-2,5-dioxopyrrol-1-yl)-3-methylquinoline-4-carboxamide(16)

White crystals, yield (66%), m.p. 255°C. ^{1}H-NMR (DMSO-d_6,δ ppm): 2.3 (s, 3H, CH_3), 2.5 (s, 4H, $2CH_2$), Ar-H: δ 7.78 (d, 2H), 7.85 (d, 2H), 8.08 (m, 1H), 8.13 (m, 1H), 8.3 (s,1H),, 10.15 (s, 1H, NH, D_2O exchangeable). MS: (□/□%) 472[M^+].Anal. calcd for $C_{21}H_{15}BrClN_3O_3$: C, 53.36; H, 3.20; N, 8.89. Found: C,53.21; H, 3.11; N, 8.92.

General procedure for synthesis of compounds 17 and 18

The suitable acid hydrazide(5 mmol) was stirred in ethanol (40 mL), containing potassium hydroxide (0.28 g; 5 mmol) for 1h until a clear solution was obtained. Carbon disulfide (1.14 g; 15 mmol) was added drop by drop to the reaction mixture with stirring and heated under reflux for 8 h, the reaction mixture was concentrated to half the volume cooled and acidified with dil. HCl and the separated product was filtered off, washed with water and recrystallized from ethanol.

4.7.1. [6-Bromo-2-(4-methylphenyl)quinolin-4-yl]-1,3,4-oxadiazole-2-thiol (17)

Yellow crystals, yield(46%), m.p. 242°C.IR (KBr, cm^{-1}): 2749 (SH).^{1}H-NMR, (DMSO-d_6,δ ppm): 2.4 (s, 3H,CH_3), Ar-H: δ 7.42(d, 3H), 8.05(d, 1H), 8.12(d, 1H), 8.25(d, 2H), 8.4(s, 1H),, 9.6(s, 1H, SH, D_2O exchangeable). MS: (□/□%) 399[M^++1], 400[M^++2]. Anal. calcd for $C_{18}H_{12}BrN_3OS$: C, 54.28; H, 3.04; N, 10.55. Found: C, 54.33; H, 3.11; N, 10.61.

[2-(3-Bromophenyl)-6-chloro-3-methylquinolin-4-yl]-1,3,4-oxadiazole-2-thiol (18)

White crystals, yield (58%), m.p. 259°C.IR (KBr, cm^{-1}): 2758 (SH).^{1}H-NMR (DMSO-d_6,δ ppm): 2.42 (s, 3H, CH_3), Ar-H: δ 7.65 (d, 2H), 7.75 (d, 2H), 7.87 (d, 1H), 8.15 (d, 1H), 8.24 (s, 1H),, 13.1 (s, 1H, SH, D_2O exchangeable).^{13}C-NMR (DMSO-d_6): 19.1, 122.9, 124.1, 125.4, 127.6, 131.0, 131.6, 131.8, 131.9, 132.1, 133.5, 139.0, 144.6, 157.1, 160.2, 178.6. MS:(□/□%) 399[M^++1], 400[M^++2]. Anal. calcd for $C_{18}H_{12}BrN_3OS$: C, 49.96; H, 2.56; N, 9.71. Found: C, 49.88; H, 2.62; N, 9.79.

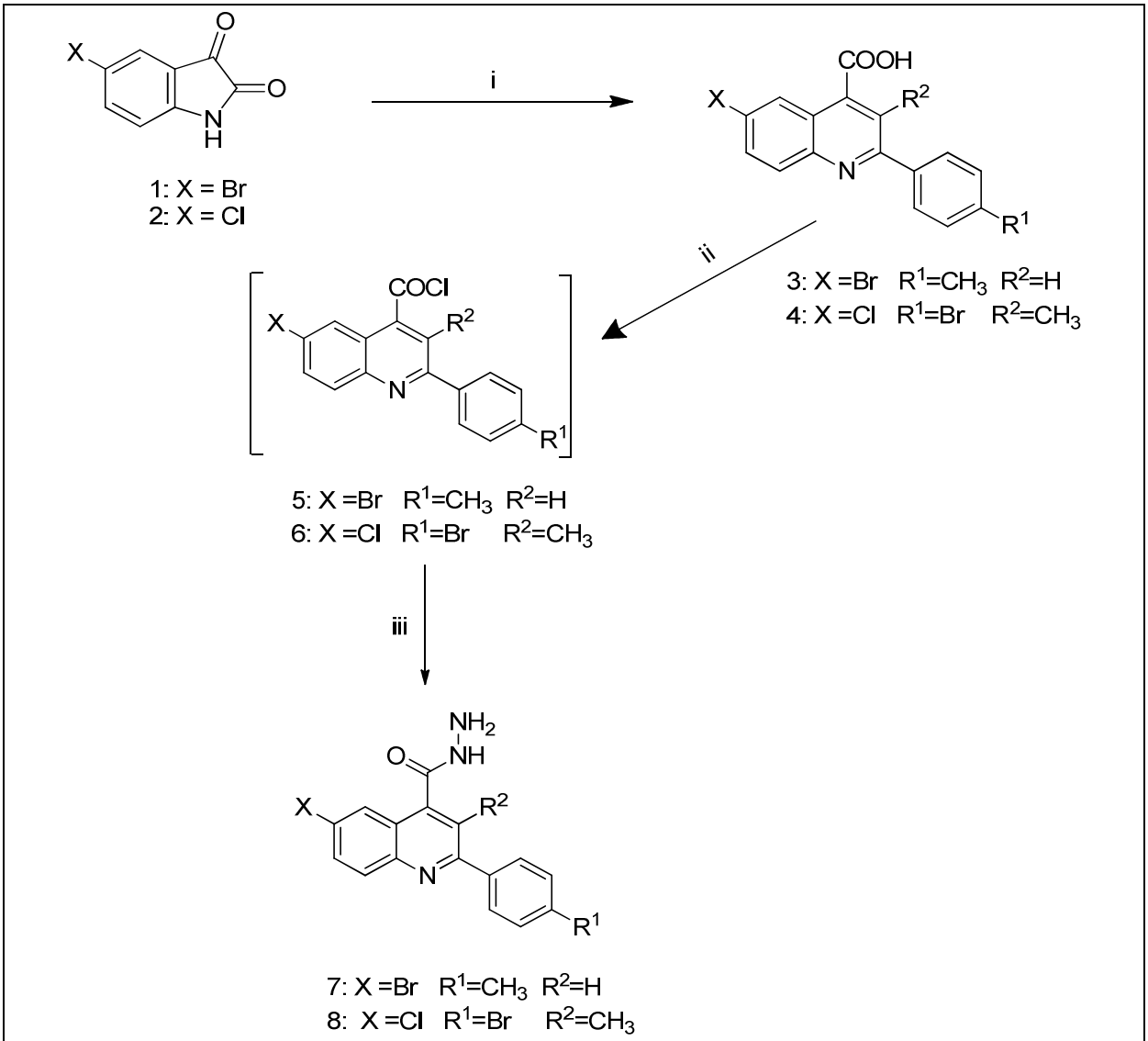
RESULTS AND DISCUSSION

2.1. Chemistry

The synthetic pathways adopted for the preparation of our new compounds are illustrated in Schemes **1** and **2**.

Starting with 5-sustituted isatins(**1**and**2)** that were prepared according to the published procedure of Sandmeyer reaction [21],quinoline-4-carboxylic acid derivatives **3**and**4** were synthetized by Pfitzinger reaction [22]in a very good yield .These two acids were reacted with thionyl chloride to prepare the corresponding acid chlorides **5** and

6which are extremely unstable intermediates, so they directly converted without separation to the corresponding acid hydrazides [6-bromo-2-(4-methylphenyl)quinoline-4-carbohydrazide **(7)**and 2-(4-bromophenyl)-6-chloro-3-methylquinoline-4-carbohydrazide **(8)**] by refluxing them with hydrazine hydratein absolute ethanol.

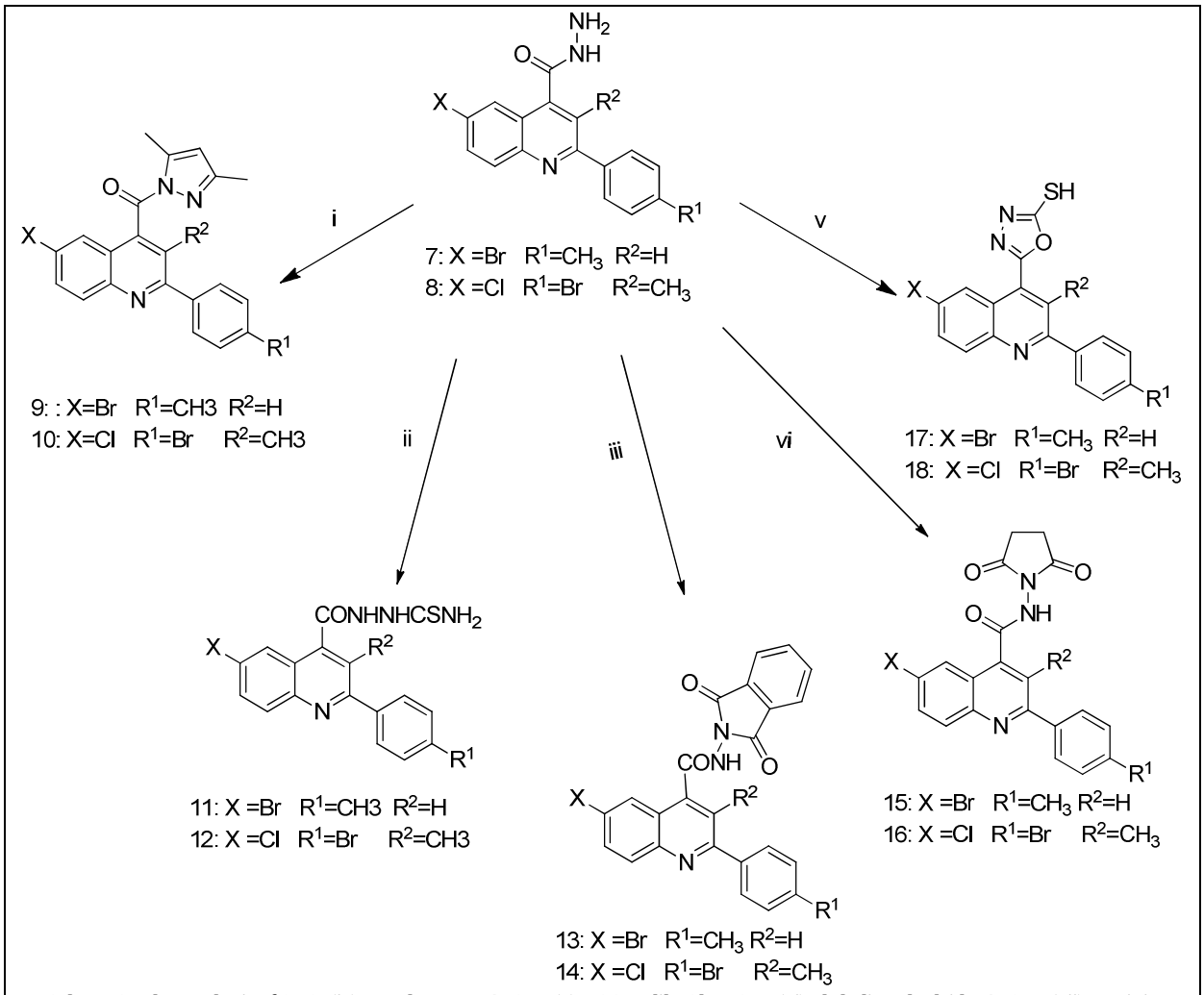


Scheme 1: The synthesis of 3–8: (i) 4-methylacetophenone or 4-bromopropiophenone, KOH, EtOH; (ii) $SOCl_2$, drops DMF, CH_2Cl_2; (iii) $NH_2NH_2.H_2O$, ab.EtOH.

Scheme 2:

The two hydrazides**7,8**were subjected to different reactions, firstly synthesis of [6-bromo-2-(4-methylphenyl)quinolin-4-yl](3,5-dimethyl-1*H*-pyrazol-1-yl)methanone **(9)** and [2-(4-bromophenyl)-6-chloro-3-methylquinolin-4-yl](3,5-dimethyl-1*H*-pyrazol-1-yl)methanone **(10)** by boiling them with acetylacetone in acetic acid. Refluxing the acid hydrazides with potassium thiocyanate and dil HCl in aqueous ethanol medium gave [6-bromo-2-(4-methylphenyl)quinoline-4-carbonyl]hydrazinecarbothioamide **(11)** and 2-(4-bromophenyl)-6-chloro-3-methylquinoline-4-carbonyl hydrazinecarbothioamide **(12).** Treating **7** and **8** with phthalic anhydride in glacial acetic acid and heating overnight gave6-bromo-*N*-(1,3-dihydro-1,3-dioxoisoindol-2-yl)-2-(4-methylphenyl) quinoline-4-carboxamide **(13)** and 2-(4-bromophenyl)-6-chloro-*N*-(1,3-dihydro-1,3-dioxoisoindol-2-yl)-3-methylquinoline-4-carboxamide**(14),** while boiling with equimolar succinic anhydride under the same conditions

resulted in **(15, 16)**. Stirring under reflux the acid hydrazide **7, 8**with potassium hydroxide and excess carbon disulfide in ethanol afforded 1,3,4 oxadiazole-2-thiol derivatives **(17, 18).**



Scheme 2: The synthesis of 9-18: (iv) acetylacetone, AcOH; (v) KSCN, dilHCl, EtOH; (vi) phthalic anhydride, AcOH; (vii) succinic anhydride, AcOH; (viii) CS_2, KOH, EtOH.

2.2. Molecular Docking

2.2.1. Methodology

Enzyme crystallographic structure:

The crystallographic VEGFR-2enzyme in complex with Lenvatinib was obtained from the RCSB Protein Data Bank (PDB entry 3WZD) [23].

Ligand preparation:

The chemical structures of most active compounds (**7**, **8** and **18**) were built using the building module. The ligand structure was charged using MMFF94 and energy minimized using MMFF force field. The lowest energy conformer was used for the docking in the binding site. Docking study was performed and data were compared with the reference ligand; Lenvatinib.

Molecular docking studies

To explain the antitumor effect of the new quinoline analogs, docking studies were performed using MOE 2007.09 program [24].The present work was based on the comparative study of docking the newly synthesized analogs in VEGFR binding pockets. In order to compare the binding affinity of the most potentially active analog, it was

docked to evaluate its degree of recognition relatively to one of the well-known antitumor agents with VEGFR inhibition affinity. Lenvatinib was recognized by interaction with three conservative crucial amino acid residues namely**; Cys919, Glu885 and Asp1046.(Figure 3)**

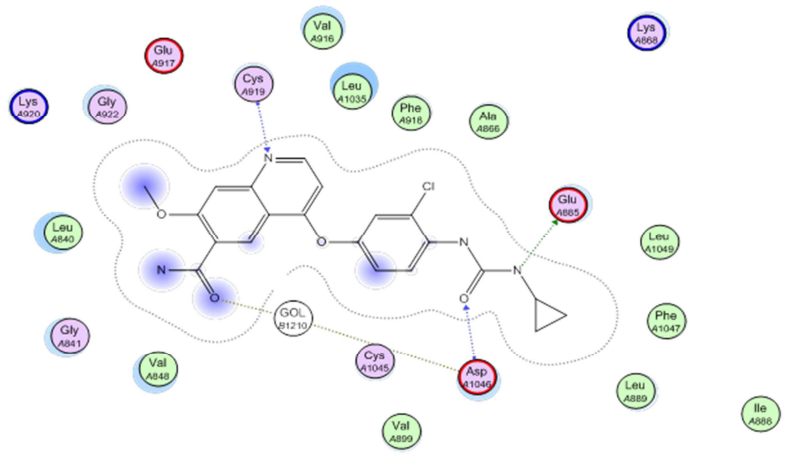


Figure 3:Docking of Lenvatinib in VEGFR-2 binding site. *N*-quinoline showed favorable recognition with Cys919 and both of Glu885 and Asp1046 favorably interacted with the *N*-CO of the urea bridge by forming strong two hydrogen bonds

Compound **7** is the most active quinoline analog that showed promising cytotoxic activity against the tumor cell lines and in agreement with the docking study results that indicated its favorable complementarity with the binding site. The 2-tolyl group performed proper hydrophobic recognition since it sets the *N*-quinoline away from interaction with any of the amino acids coating the binding pocket. However, the carbonyl oxygen was laid in proper configuration that permits the interaction with Gly846 forming strong hydrogen bond. Terminal chain of the hydrazide group was held by two hydrogen bonds with the crucial amino acids Asp1046 and Arg1032**. (Figure 4)**

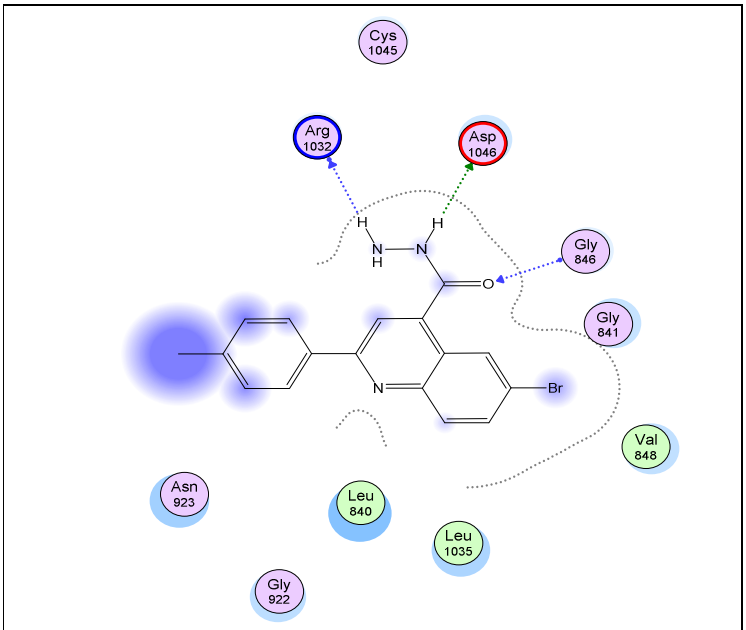
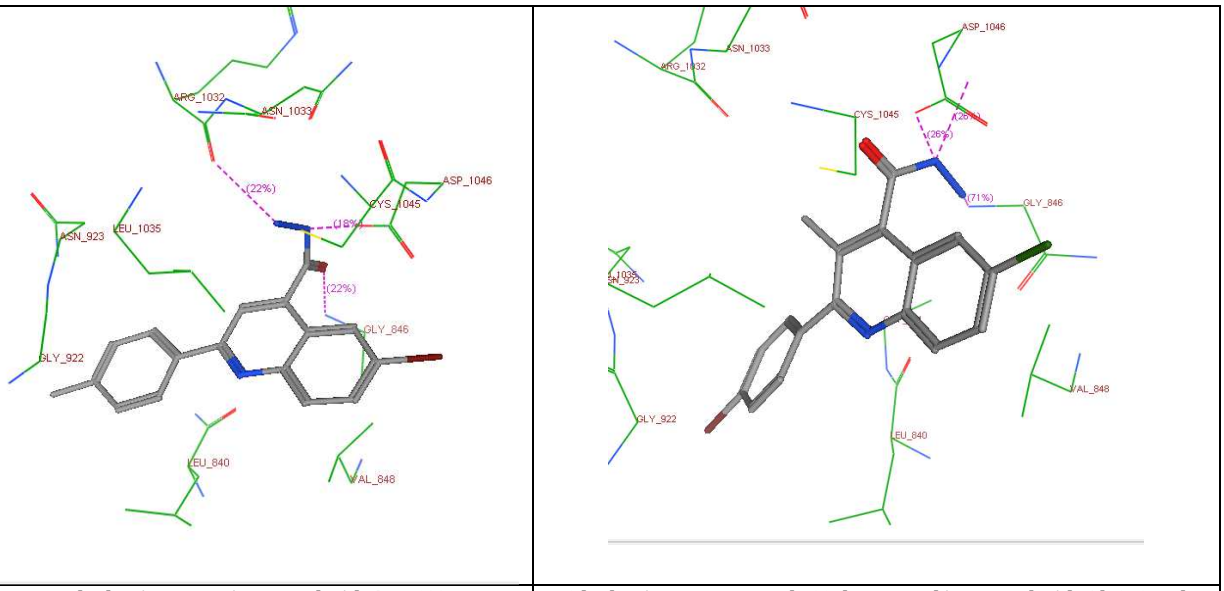


Figure 4: Binding models of compound 7 docked into the active site of VEGFR2. Hydrogen bonds were showed in blue dashed lines

Remarkably, Compound **8** is substituted with 3-methyl group that forces the 4-substituted hydrazide group to be conformationally strayed to the opposite direction avoiding the steric effect. This new confirmation leads to the presence of two different patterns of recognition with the surrounding pocket residues **(**in comparison with compound **7). (Figure 5)**



7 hydrazino group interacted with Arg1032	8 hydrazino group turned 180 degree and interacted with Gly846 and Asp1046

Figure 5:Effect of3-methyl group on hydrazino group interactions

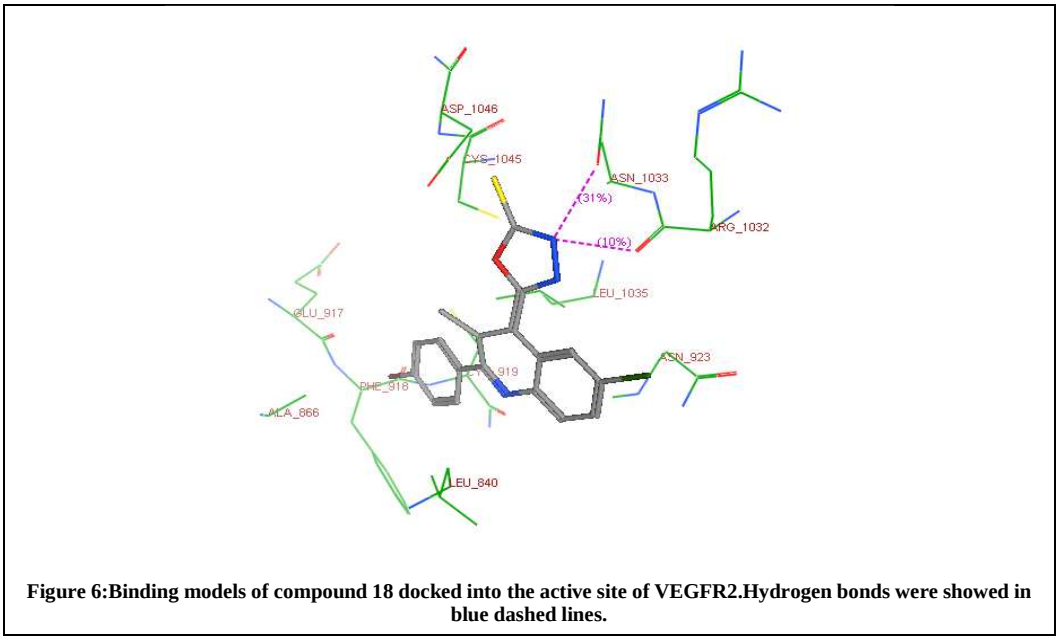


Figure 6:Binding models of compound 18 docked into the active site of VEGFR2.Hydrogen bonds were showed in blue dashed lines.

Compound **18** is one of the most active analogs that showed favorable cytotoxic activity against the five cell lines. Even the *N*-quinoline was set away due to the 4-bromophenyl moiety, however the presence of the oxazole ring augment the complementarity between the two *N*-heterocyclic ring that strongly interact with Asn1033 and Arg1032 forming two hydrogen bonds. Additionally; Asp-1046 is one of the conserved amino acids at the binding pocket

performing favorable interaction with the thiol group of the oxazole ring. The overall docking performance of compound 18 was promising to consider it as one of the potential VEGFR inhibitors. **(Figure 6)**

3. Biological screening(antitumor activity):
All synthesized quinoline compounds were evaluated for their antitumor activities against five human tumor cell lines namely; hepatocellular carcinoma (HePG-2), Colorectal carcinoma (HCT-116), mammary gland (MCF-7), Human prostate cancer (PC3) and Epithelioid Carcinoma (Hela). The results are presented in **table 1**. Compounds**7** and **8** exhibited the best activity (very strong) against the five tested cell lines (**7** showed superior activity than 5-FU against HePG2 and PC3). Compounds **11, 12, 17** and **18** showed strong activities against all tested cell lines. Compound **14** exhibited strong activity against HePG2 and HCT-116, while moderately affected other cell lines. Compounds **9, 10** and **13** showed moderate activity on the five cell lines, compound **15** also had moderate effects on the cell lines except PC3 (weak). Compound **16** had weak activity against most cell lines, but still better than that of the starting compounds **3** and **4**.

The cell lines were obtained from ATCC *via* Holding company for biological products and vaccines (VACSERA), Cairo, Egypt, 5-fluorouracil was used as a standard anticancer drug for evaluation.

Chemical reagents:
The reagents are RPMI-1640 medium, DMSO, MTT, 5-fluorouracil (Sigma Co, USA) and Fetal Bovine serum (GIBCO, UK).

MTT assay
The cell lines cited above were used to verify the cytotoxic effects of our compounds on cell growing using the MTT assay. The principle of this assay is the transformation of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells [25, 26]. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. The antibiotics added were 100µg/ml streptomycin and 100 units/ml penicillin at 37□ C in a 5% Co_2 incubator. The cell lines were planted in a 96-well plate at a density of 1.0x104 cells/ well at 37□ C for 48 h under 5% Co_2[27].After incubation the cells were handled with several concentrations of the synthesized compounds and incubated for 24 h. After that, 20 µl of MTT solution at 5mg/ml was included and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is supplemented into each well to dissolve the purple formazan made. The colorimetric assay is calculated and registered at absorbance of 570 nm using a plate reader (EXL 800 USA).

Table 1 Cytotoxic activity of the synthesized compounds against human tumor cells

	In vitro Cytotoxicity IC_{50}(µg/ml)•				
	HePG2	**HCT-116**	**MCF-7**	**PC3**	**Hela**
5-FU	7.9±0.28	5.3±0.17	5.4±0.20	8.3±0.35	4.8±0.21
3	64.0±4.13	83.7±4.82	>100	78.2±5.46	91.5±5.87
4	58.8±3.87	65.5±4.36	90.4±6.52	82.0±5.14	>100
7	5.6±0.25	5.4±0.30	8.1±0.90	7.4±0.42	6.8±0.47
8	9.3±0.49	8.7±0.34	8.9±0.96	8.1±0.73	7.7±0.63
9	40.5±2.77	39.1±2.32	52.3±3.89	26.2±2.06	19.8±1.85
10	33.6±2.51	25.5±1.96	44.3±3.47	35.1±2.45	44.7±3.71
11	13.4±1.06	12.9±1.14	18.3±1.68	10.4±0.98	9.2±0.82
12	12.3±0.96	11.0±1.03	15.6±1.27	13.8±1.34	17.4±1.43
13	19.9±1.34	21.8±1.40	30.5±2.83	29.8±2.41	32.6±2.78
14	17.4±1.40	16.3±1.15	28.4±2.16	20.4±1.86	18.7±1.72
15	48.2±3.81	26.9±1.89	33.4±2.53	59.7±3.67	46.0±3.41
16	68.2±4.35	41.6±3.62	86.8±5.14	70.6±4.68	53.0±3.80
17	14.7±1.23	15.8±1.23	19.2±1.65	12.5±1.17	13.2±1.56
18	10.3±0.88	9.7±0.87	14.4±1.12	12.2±1.32	11.4±1.30

*•**IC50 (µg/ml)**: 1 – 10 (very strong).>10 – 20 (strong). >20 – 50 (moderate). >50 – 100 (weak) and above 100 (non-cytotoxic).*

CONCLUSION

Most of the newly synthesized compounds showed promising activity against HePG2, HCT-116, MCF-7, PC3 and Hela cell lines in comparison to 5-FU as reference agent. A strong correlation between biological screening results and molecular modeling was proven. From the achieved results, we can conclude that:**1)**The incorporation of

hydrazide moiety (-CONHNH) at position 4 of quinoline ring is important for exerting the cytotoxic activity of these compounds, so compounds 7, 8, 11 and 12 have a very strong activity against the five cell lines**2)** Methylation of quinoline ring at position 3 slightly decreases the activity as in compounds 8.**3)**Converting CONHNH to oxadiazole ring in compounds 17 and 18 preserves the strong activity on most cell lines. **4)**However, in compounds 13 and 14the presence of phthalimide moiety attached to (4- CONH)decreases their activity to moderate in most cell line. Finally these results would be a promising study for future more potent antitumor quinoline drugs.

Acknowledgements
The authors would like to thank Faculty of Pharmacy, Mansoura University for funding this work and thanks to Dr. Abdelbasset A. Farahat, pharmaceutical organic chemistry dept, faculty of pharmacy, Mansoura University for helping in the interpretation of the NMR spectra.

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