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Original Research Article

Synthesis of new hybrid quinazoline compounds as antiproliferative agents for breast and colon cancer treatment

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Abstract

Purpose: *To evaluate newly synthesized fuoryl quinazoline derivatives for antitumor efficacy.*
Methods: *Fuoryl quinazoline derivatives were synthesized and the structures of the synthesized compounds were characterized using standard techniques. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) technique was used to assess the anti-proliferative properties of the synthesized derivatives in vitro.*
Results: *All quinazoline compounds displayed cytotoxic activity against breast and colon cancer cell lines to varying degrees. Compound IXa with acetohydrazide moiety was the most effective on MCF7 and HCT116 cell lines, with half-maximal inhibitory concentration (IC_{50}) values of 16.70 and 12.54 µM, respectively.*
Conclusion: *N'-benzylidene-2-((2-(furan-2-yl) quinazolin-4-yl) oxy) acetohydrazide IXa showed the strongest anti-proliferative activity against MCF-7 and HCT116 human cancer cell lines.*

Keywords: *Quinazoline, Antitumor, Acetohydrazide, Carbothioamide*

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INTRODUCTION

Cancer is a malignant, life-threatening disease that ranks second only to cardiovascular diseases in terms of morbidity and mortality and is expected to be the leading cause of death worldwide in the future [1]. Because of their side effects, systemic toxicity, and the resistance of current non-selective cytotoxic chemotherapies, the identification of effective, safe, and novel anticancer agents remains an important research field [2]. Nitrogenous heterocyclic compounds are the most privileged chemical molecules and have shown potential anticancer effects against a panel of human cancer cell lines [3-11]. Quinazolines are nitrogen-containing heterocyclic scaffolds with a broad variety of biological activities, such as antitubercular [12], anti-inflammatory [13], antimicrobial [14] and anticancer activities [15].

The FDA has authorized many quinazoline derivatives as anticancer drugs, including Erlotinib, Lapatinib, Gefitinib, and Caneratinib

[16,17]. Thiourea functional groups have been shown to enhance anticancer activity [18]. In view of the aforementioned rationale and in continuation of the research program regarding the synthesis of new and safer anticancer agents [19-21], this study evaluates a new series of quinazoline hybridized with a thiourea moiety in order to obtain new quinazoline derivatives with high anticancer activity (Figure 1).

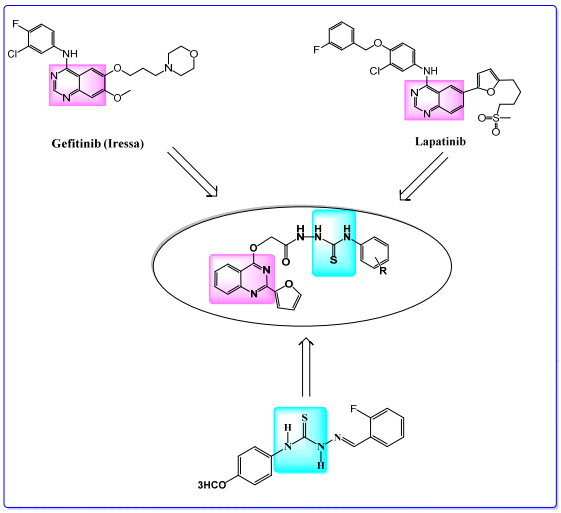


Figure 1: Reported antitumor drugs containing quinazoline moiety or thiourea moiety and our designed compounds

EXPERIMENTAL

Chemistry

Melting points were determined using an Electrothermal Stuart 5MP3 apparatus. The IR spectra for KBr disc were recorded using a Perkin Elmer-9712 spectrometer. A Bruker NMR spectrometer-400 was utilized to perform routine NMR measurements. The mass spectra were analyzed using a Finnigan Mat SSQ 7000 EI apparatus. Compound III was prepared according to reported procedure [22].

Synthesis of compounds VIIIa-d

A mixture of VII (0.01 mol) and appropriate isothiocyanate (0.01 mol) were refluxed in ethanol (20 mL) for 6 h. Then the mixture was cooled, filtered, and crystallized from ethanol.

Cancer cell line screening

In vitro cytotoxicity of synthesized compounds was assessed using the conventional MTT technique [21]. In this work, two human tumor cell lines, MCF-7 and HCT-116, were tested using doxorubicin as reference drug. The cells were grown in RPMI-1640 media supplemented with 10 % fetal bovine serum and antibiotics (100 unit/mL penicillin and 100 g/mL streptomycin) and seeded in 96-well plate for 48 h in a 37 ℃/5 % CO_2 incubator. Following incubation, cells were treated with various concentrations of the synthesized compounds and incubated for another 24 h. Thereafter, the MTT solution was added and plates were incubated for another 4 h. To dissolve the produced purple formazan, DMSO was added to each well and the absorbance was measured spectrophotometrically at 570 nm. The proportion of relative cell viability was determined and Table 1 summarizes the results for the IC_{50} values of the active compounds.

RESULTS

Spectral characteristics of the synthesized compounds

2-(2-((2-(furan-2-yl)quinazolin-4-yl)oxy)acetyl)-N-phenylhydrazine-1-carbothioamide (V111a)

Yield 69 %, mp 214 – 216 ℃, IR (KBr, cm^{-1}): 3380, 3372, 3240 (3NH), 1680 (CO). MS: m/z = 419; Anal for $C_{21}H_{17}N_5O_3S$; Calcd C, 60.13; H, 4.09; N, 16.70; Found: C, 60.10; H, 4.11; N, 16.75. ^{1}H NMR ($DMSOd_6$): δ 4.45 (2H, s, OCH_2), 7.18 – 7.62 (12H, m, ArH), 8.12 (1H, s, NH), 9.31 (1H, s, NH), 10.10 (1H, s, NH-ph). ^{13}C NMR ($DMSOd_6$) δ 70, 107, 111, 119, 120, 121, 122, 123, 125, 127, 128, 129, 134, 138, 142, 151, 154, 160, 166, 181, 183.

N-(2-fluorophenyl)-2-(2-((2-(furan-2-yl)quinazolin-4-yl)oxy)acetyl)hydrazine-1-carbothioamide (VIIIb)

Yield 73 %, mp 190 – 192 ℃, IR (KBr, cm^{-1}): 3375, 3370, 3243 (3NH), 1683 (CO). MS: m/z = 437; Anal for $C_{21}H_{16}FN_5O_3S$; Calcd C, 57.66; H, 3.69; N, 16.01; Found: C, 57.69; H, 3.65; N, 16.03. ^{1}H NMR ($DMSOd_6$): δ 4.40 (2H, s, OCH_2), 7.20 – 7.59 (11H, m, ArH), 8.10 (1H, s, NH), 9.25 (1H, s, NH), 10.12 (1H, s, NH-ph). ^{13}C NMR ($DMSOd_6$) δ 69, 107, 112, 119, 120, 122, 123, 124, 126, 127, 128, 129, 133, 137, 142, 150, 154, 161, 165, 181, 182.

2-(2-((2-(furan-2-yl)quinazolin-4-yl)oxy)acetyl)-N-(4-methoxyphenyl)hydrazine-1-carbothioamide (VIIIc)

Yield 80 %, mp 217 – 219 ℃, IR (KBr, cm^{-1}): 3387, 3368, 3246 (3NH), 1685 (CO). MS: m/z = 449; Anal for $C_{22}H_{19}N_5O_4S$; Calcd C, 58.79; H, 4.26; N, 15.58; Found: C, 58.74; H, 4.20; N, 15.55. ^{1}H NMR ($DMSOd_6$): δ 3.84 (3H, s, OCH_3),

4.45 (2H, s, OCH_2), 7.10 (2H, d, *J* = 8.2 Hz, ArH), 7.21 – 7.57 (7H, m, ArH), 7.60 (2H, d, *J* = 8.0 Hz, ArH), 8.01 (1H, s, NH), 9.36 (1H, s, NH), 10.15 (1H, s, NH-ph). ^{13}C NMR ($DMSOd_6$) δ 55, 68, 112, 118, 120, 121, 123, 125, 128, 129, 132, 136, 142, 151, 154, 159, 160, 165, 182, 185.

2-(2-((2-(furan-2-yl)quinazolin-4-yl)oxy)acetyl)-N-(4-(trifluoromethyl)phenyl)hydrazine-1-carbothioamide (VIIId)

Yield 83 %, mp 187 – 189 ℃, IR (KBr, cm^{-1}): 3376, 3360, 3237 (3NH), 1679 (CO). MS: m/z = 487; Anal for $C_{22}H_{16}F_3N_5O_3S$; Calcd C, 54.21; H, 3.31; N, 14.37; Found: C, 54.25; H, 3.35; N, 14.31. ^{1}H NMR ($DMSOd_6$): δ 4.41 (2H, s, OCH_2), 7.24 (2H, d, *J* = 8.0 Hz, ArH), 7.35 – 7.64 (7H, m, ArH), 7.73 (2H, d, *J* = 8.0 Hz, ArH), 8.15 (1H, s, NH), 9.40 (1H, s, NH), 10.37 (1H, s, NH-ph). ^{13}C NMR ($DMSOd_6$) δ 68, 108, 113, 118, 119, 121, 123, 127, 128, 129, 130, 131, 135, 140, 145, 154, 160, 165, 182, 185.

Synthesis of compounds IXa-h

A mixture of **VII** (0.02 mol) and appropriate aldehyde (0.02 mol) in ethanol (30 mL) was refluxed for 9 h, filtered and crystallized from ethanol.

N'-benzylidene-2-((2-(furan-2-yl)quinazolin-4-yl)oxy)acetohydrazide (IXa)

Yield 65 %, mp 175 – 177 ℃, IR (KBr, cm^{-1}): 3380, (NH), 1683 (C=O). MS: m/z = 372; Anal for $C_{21}H_{16}N_4O_3$; Calcd C, 67.73; H, 4.33; N, 15.05; Found: C, 67.69; H, 4.30; N, 15.00.^{1}H NMR ($DMSOd_6$): 4.37 (2H, s, OCH_2), 7.25 – 7.70 (12H, m, ArH), 8.34 (1H, s, CHN), 10.80 (1H, s, NH). ^{13}C NMR ($DMSOd_6$) δ 69, 112, 117, 119, 120, 121, 125, 126, 127, 129, 131, 132, 134, 136, 142, 144, 150, 154, 163, 172, 180.

N'-(2-fluorobenzylidene)-2-((2-(furan-2-yl)quinazolin-4-yl)oxy)acetohydrazide (IXb)

Yield 68 %, mp 179 – 181 ℃, IR (KBr, cm^{-1}): 3386, (NH), 1688 (C=O). MS: m/z = 390; Anal for $C_{21}H_{15}FN_4O_3$; Calcd C, 64.61; H, 3.87; N, 14.35; Found: C, 64.66; H, 3.85; N, 14.30.^{1}H NMR ($DMSOd_6$): 4.35 (2H, s, OCH_2), 7.20 – 7.62 (11H, m, ArH), 8.30 (1H, s, CHN), 10.83 (1H, s, NH). ^{13}C NMR ($DMSOd_6$) δ 67, 111, 115, 117, 119, 121, 125, 126, 127, 129, 132, 135, 136, 142, 146, 150, 157, 159, 163, 170, 181.

Structure of the compounds

The reaction of anthranilic acid **I** and fuorylchloride **II** resulted in the amide analog **III** which was refluxed in acetic anhydride to obtain 2-(furan-2-yl)-4H-benzo[d][1,3]oxazin-4-one **IV** [22] . When compound **IV** reacted with formamide, compound **V** was formed [22] (Figure 2).

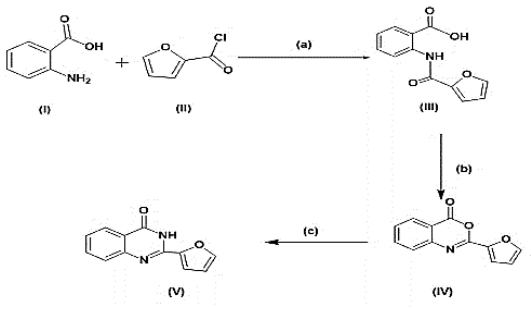
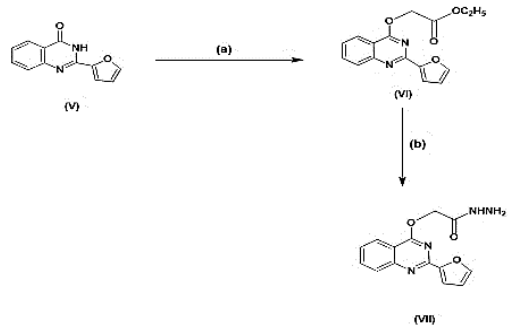


Figure 2: Reagents and conditions: (a) Pyridine, reflux, 3 h; (b) AC_2O, reflux, 2 h, (c) $HCONH_2$ reflux 5 h

Compound **VI** was produced by the reaction of **V** with ethyl chloroacetate in the presence of anhydrous potassium carbonate. Refluxing compound **VI** with hydrazine hydrate yielded hydrazide VII (Figure 3).



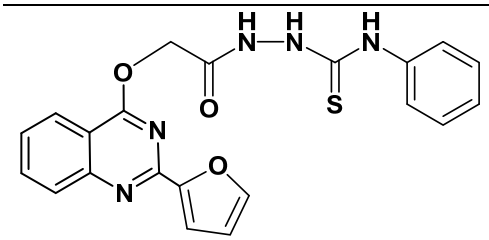
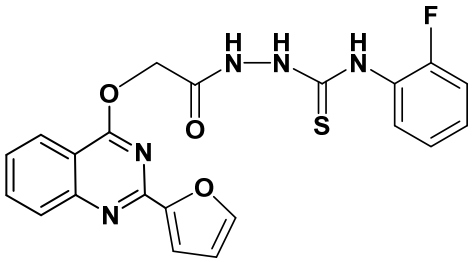
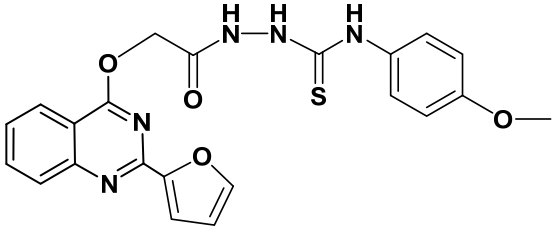
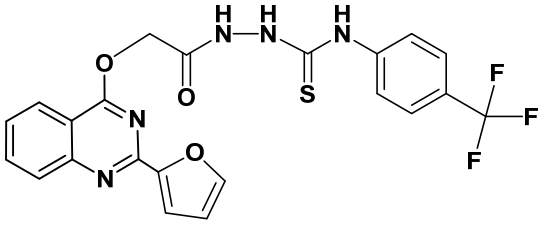
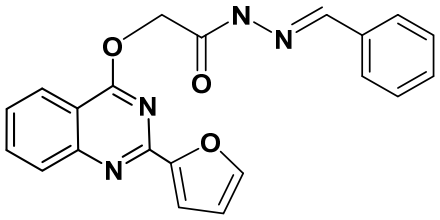
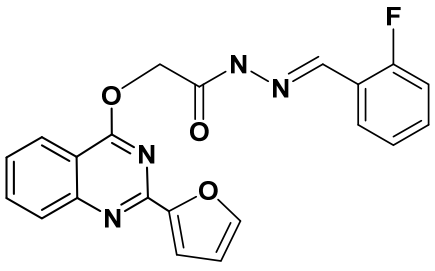
Scheme 3: Reagents and conditions: (a) Ethyl chloroacetate, potassium carbonate, acetone, reflux,10 h; (b) NH_2NH_2, EtOH, reflux, 3 h

The reaction of hydrazide **VII** with appropriate isothiocyanate in ethanol yields compounds **VIIa-d**. Schiff bases **IXa,b** was synthesized by reacting **VII** with the appropriate aldehyde in the presence of ethanol (Figure 4).

Anticancer activity

The cytotoxic effect of two cell lines, HCT116 and MCF-7, was tested *in vitro* and all compounds showed promising cytotoxicity. N'-benzylidene-2-((2-(furan-2-yl)quinazolin-4-yl)oxy)acetohydrazide **IXa** was the most potent compound on all cell lines tested, with IC_{50} values ranging from 12.54 to 16.70 µM (Table 1, Figure 5 and Figure 6).

Table 1: *In vitro* antitumor activity of all compounds on breast (MCF-7) and colon (HCT116) cancer cell lines

Structure	Comp No	MCF-7	HCT116
		IC_{50} *(mean ± SD) µM*	
	VIIIa	38.18±0.005	36.15±0.007
	VIIIb	42.9±0.01	35.97±0.006
	VIIIc	100.73±0.003	71.86±0.009
	VIIId	95.12±0.007	76.92±0.01
	IXa	16.7±0.009	12.54±0.004
	IXb	159.8±0.01	47.59± 0.003
	Dox	**4.67±0.004**	**3.85 0.002**

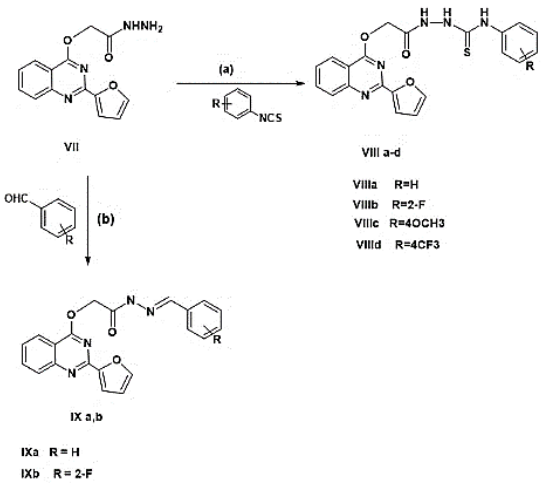


Figure 4: Reagents and conditions: (a) Ethanol, reflux, 6 h; (b) Ethanol, reflux, 9 h

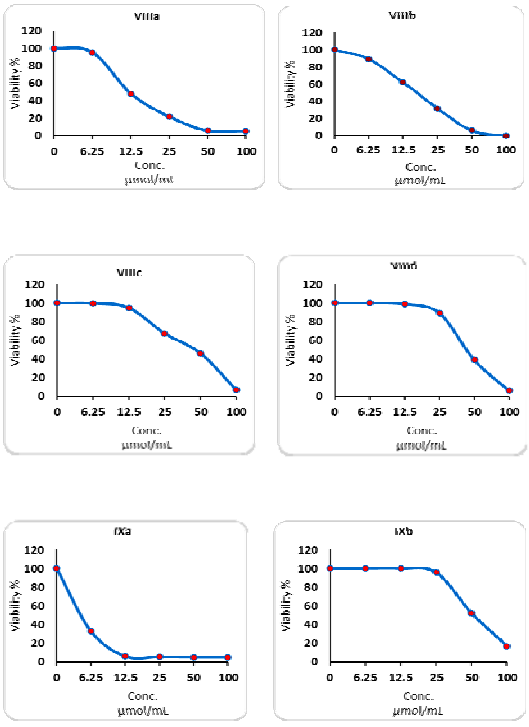


Figure 5: Cell viability of all synthesized compounds against MCF7 cell line

DISCUSSION

Using the sulphorhodamine-B assay technique, a new series of fuoryl quinazoline compounds have been synthesized and evaluated for their antiproliferative activities on two cancer cell lines, breast (MCF7) and colon (HCT116) with all data expressed as IC_{50} values.

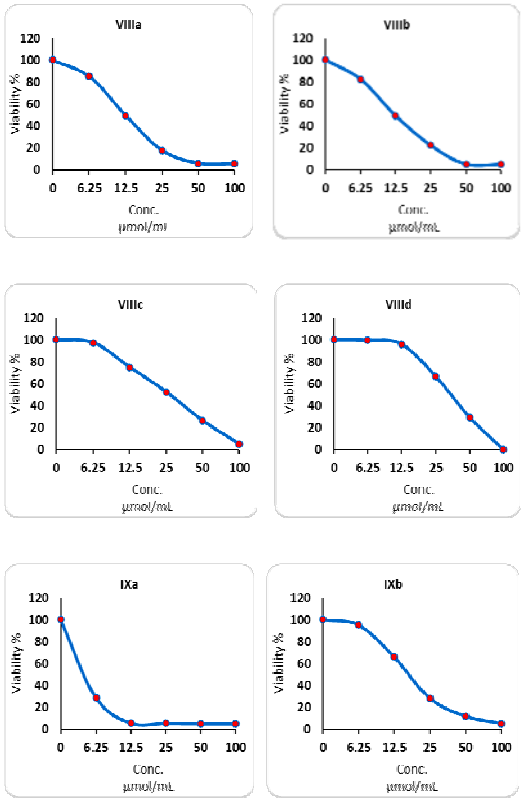


Figure 6: Cell viability of all synthesized compounds against HCT116 cell line

Compounds **IXa** with a benzylidene-acetohydrazide moiety was the most potent against (MCF7) and (HCT116) cancer cells with IC_{50} between 16.70 and 12.54 µM respectively. On the other hand, substitution with 2-fluoro benzylidene-acetohydrazide decreased antiproliferative activity on both cell lines at IC_{50} values of 159.8 and 47.59 µM respectively. Compounds **VIIIa** and **VIIIb** with N-phenylhydrazine-1-carbothioamide and 2-fluoro N-phenylhydrazine-carbothioamide show moderate cytotoxic activity on (MCF7) and (HCT116) cancer cells. Substitution with 4 methoxy or 4-(trifluoromethyl) phenylhydrazine-carbothioamide markedly decrease anticancer activity (Table 1).

CONCLUSION

Novel quinazoline derivatives containing fuoryl moiety have been synthesized and evaluated *in vitro* against a human breast cancer cell line (MCF-7) and a colon cancer cell line (HCT116). Compound IXa is the most effective against both cancer cell lines, with IC_{50} values of 16.70 and 12.54 µM. Further toxicity and safety studies on the compounds *in vivo* are required.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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