

New series of 4(3*H*)-quinazolinone derivatives: syntheses and evaluation of antitumor and antiviral activities

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Abstract New series of 3-(3-trifluoromethylphenyl)-6-iodo-4(3*H*)-quinazolinone derivatives bearing thiosemicarbazones, pyrazoles, azomethine moieties at C-2 were synthesized. The obtained products were screened for their expected anticancer activity against; human liver cancer cell line (HepG2), breast cancer cell line (MCF-7) and human lung adenocarcinoma epithelial cell line (A549) tumor cell lines. Cytotoxicity of the synthesized compounds showed good IC₅₀ for some products in comparison with the standard drug, doxorubicin. On the other hand, antiviral activity of the synthesized products against H₅N₁ showed moderate to weak activity compared to Zanamivir reference drug.

Keywords 4(3*H*)-Quinazolinones · Thiosemicarbazones · Pyrazoles · Schiff's bases · Antitumor activity · Antiviral activity

Introduction

Cancer is one of the major worldwide health problems (Eckhardt 2002; Zhou et al. 2017). Over the span of recognizing different compounds which may serve as antitumor agents, quinazolinone derivatives have been identified as a new class of cancer chemotherapeutic agents with

significant therapeutic efficacy against some solid tumors (Khatib et al. 2015; Al-Omary et al. 2010; Al-Rashood et al. 2006).

Influenza A is a negative-strand RNA virus. It infects the upper and lower respiratory tracts. It also causes substantial morbidity and mortality annually (Ford and Grabenstein 2006). Avian H₅N₁ viruses can infect the lower respiratory tract, causing hyper-cytokemia, and increased tissue damage (van Riel et al. 2006). Avian H₅N₁ viruses have resulted in the culling of hundreds of millions of birds and awareness of more human cases (Webster and Govorkova 2006). So, many synthetic compounds have been tried as potential inhibitors of the avian H₅N₁ viruses where, some of them showed the capacity to hinder viral replication, for example, zanamivir and oseltamivir. Since rise of new drug resistant mutant variations of flu viruses is normal occasion, the requirement for option hostile to flu drugs is an earnest worldwide need (Li et al. 2006).

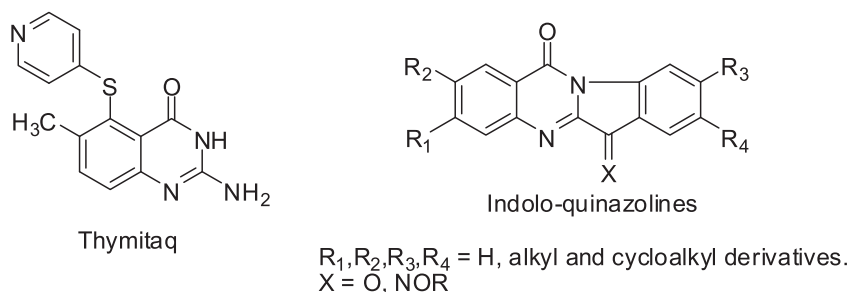
Interest in quinazolinone derivatives as anticancer agents has further increase since the discovery of Raltitrexed and Thymitaq (Fig. 1), which were proved to be thymidylate enzyme inhibitors (Smaill et al. 2000; Wissner et al. 2000). Some indolo-quinazolinone derivatives were characterized by their activity towards a panel of human cancer cell lines (Sharma et al. 2002). Also, 4-anilinoquinazolines represents a class of antitumor drugs (Smaill et al. 2000; Wissner et al. 2000). They inhibit the epidermal growth factor receptor (EGFR) tyrosine kinase over expression through the inhibition of EGFR autophosphorylation and EGF stimulated signal transduction (Kersemaekers et al. 1999; Maurizi et al. (1996). Furthermore, quinazolines exert their antitumor activity through inhibition of the DNA repair enzyme system (Bridges et al. 1996; Wakeling et al. 1996; Griffin et al. 1998). Moreover, 4(3*H*)-quinazolinone derivatives were reported as very significant heterocyclic compounds, and commonly used

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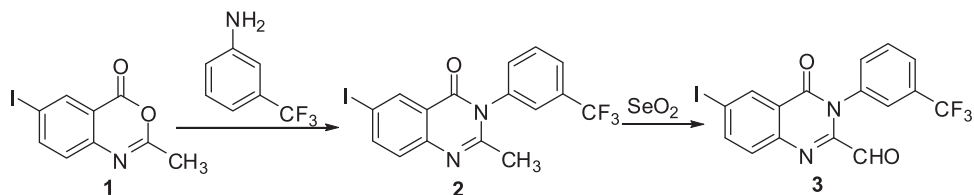
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Fig. 1 Some quinazolinone derivatives as anticancer agents



Scheme 1 Synthesis of aldehyde **3**



as potential biological and pharmaceutical agents, such as anti-inflammatory, analgesic, antimicrobial, and anti-tumor (Abbas et al. 2016; Aly et al. 2010; Farag et al. 2013; Ammar et al. 2011; El-Bayouki et al. 2011).

The literature survey exposed that many heterocyclic moieties bearing halogen atom have attracted attention due to the ability of halogen to act as polar hydrogen or hydroxyl-mimic (Abbas et al. 2013). In our previous, series of 6-iodo-4(3*H*)-quinazolinone derivatives was synthesized and characterized by their promising biological activity. Where, incorporation of substituent at C-2 such as thiosemicarbazone moiety enhanced the biological activity of the quinazolinone system (Aly et al. 2010; Farag et al. 2013).

So, in continuation of our studies, our research program was directed towards the synthesis of newer derivatives with promising their biological properties. Thus, a novel series of quinazolinone derivatives bearing iodine atom at position-6, 3-trifluoromethyl phenyl at position-3, and different substituents at position-2 were synthesized as a trial to get more powerful biologically active compounds.

Results and discussion

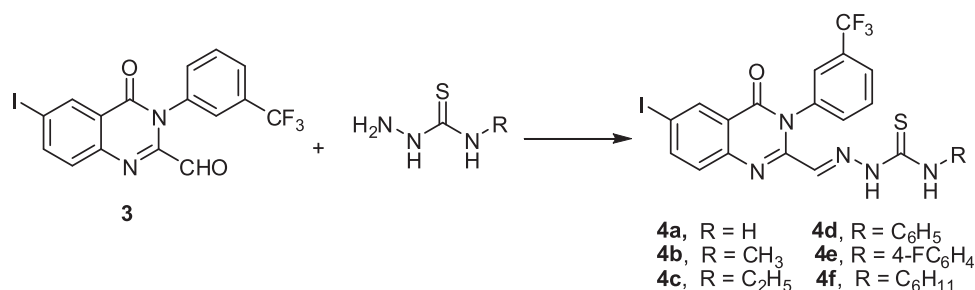
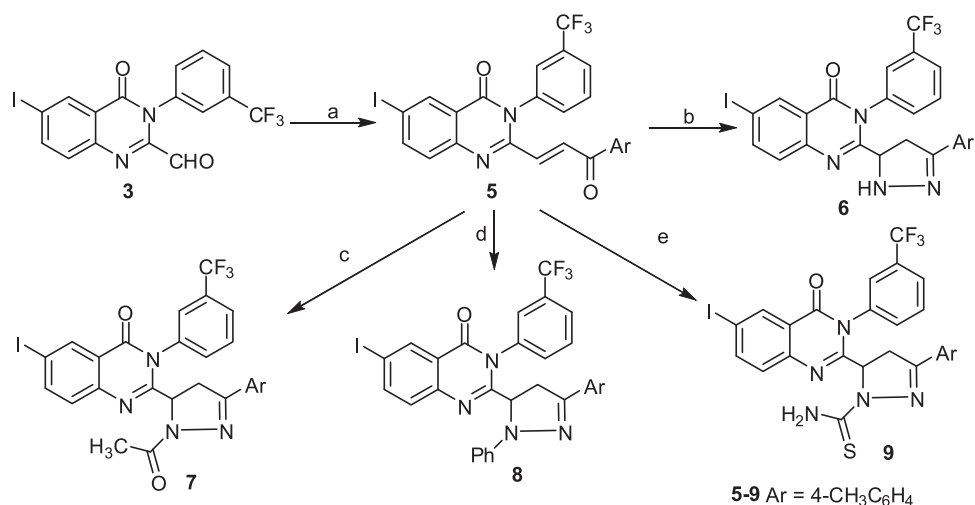
Chemistry

3,1-Benzoxazinone **1** condensed readily with 3-trifluoromethylaniline to afford the desired product 2-methyl-4(3*H*)-quinazolinone **2** in high yield. On further reaction oxidation the methyl group to a formyl group utilizing SeO_2 was carried out. The new 4(3*H*)-quinazolinone-2-carboxaldehyde **3** was prepared (Scheme 1). The oxidizing

agent acts as an electrophile. The reaction took place presumably through the formation of two intermediates to give the proposed structure **3** as has been reported in our previous work (Aly et al. 2010; Farag et al. 2013). IR spectrum of the aldehyde **3** revealed the carbonyl peaks at: 1719 ($\text{C}=\text{O}$ formyl), 1687 ($\text{C}=\text{O}$ quinazolinone). Furthermore, its ^1H NMR spectrum displayed signal at: $\delta = 9.43$ p.p.m. characteristic for the proton of formyl group.

Due to the potential biological value of thiosemicarbazone derivatives, it was noticed that their biological activity depending on the parent aldehyde or ketone (El-Sharief et al. 2013). Thus, the thiosemicarbazone derivatives **4a–f** were synthesized via the reaction of 4(3*H*)-quinazolinone-2-carboxaldehyde **3** with some *N*-substitutedthiosemicarbazide derivatives (Scheme 2) in good yields. Their IR spectra showed bands at $3437\text{--}3127\text{ cm}^{-1}$ and $1687\text{--}1681\text{ cm}^{-1}$ regions attributed to the NH and $\text{C}=\text{O}$ groups, respectively. ^1H NMR spectra of products **4** revealed signals around δ : 11.80 p.p.m. (D_2O -exchangeable) for NH group, with appearance of the aromatic protons in their expected positions at δ : 6.68–8.14 p.p.m. ^1H NMR spectrum of **4c** was characterized by the presence of triplet and quartet signals at δ : 1.01 and 3.40 p.p.m., respectively, due to ethyl protons. The mass spectra of the derivatives **4a–f** revealed molecular ion peaks which were in accordance to their structures. Scheme 3.

Biological activity of pyrazole moiety encouraged us to synthesize a new series of 4(3*H*)-quinazolinone derivatives containing this moiety (Farag et al. 2015). The presence of the formyl group in the quinazolinone derivative **3** provide it as a precursor for synthesizing chalcones (α,β -unsaturated ketones). Thus, the Claisen-Schmidt condensation of

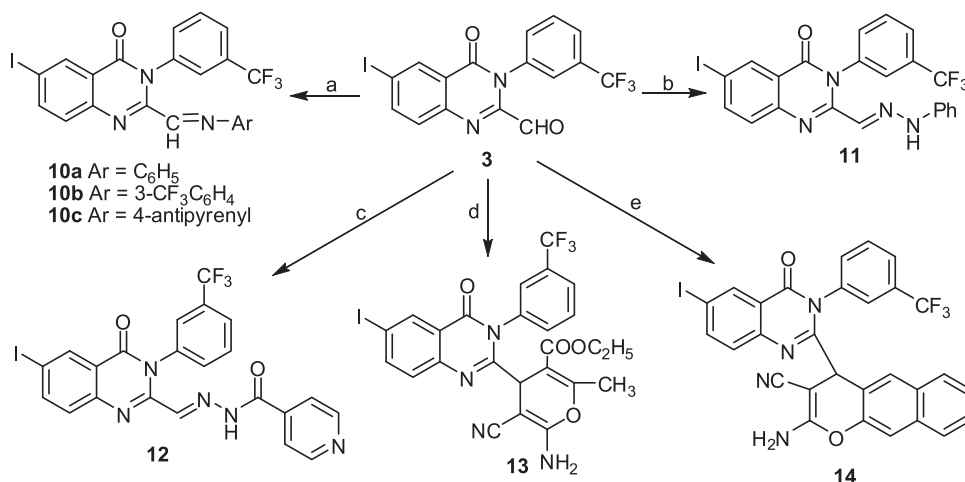
Scheme 2 Synthesis of thiosemicarbazones **4a–f****Scheme 3** Synthesis of chalcone **5** and pyrazole derivatives **6–9**. Reagents and conditions: (a) 4-methyl acetophenone, NaOH, aqueous methanol, reflux 1 h; (b) hydrazine hydrate, ethanol, reflux 4 h; (c) hydrazine hydrate, glacial acetic, reflux 8 h; (d) phenylhydrazine, ethanol, NaOH, reflux 6 h; (e) thiosemicarbazide, ethanol, NaOH, reflux 10 h

quinazolin-2-aldehyde **3** with 4-methylacetophenone in ethanolic sodium hydroxide furnished the chalcone derivative **5**. Cyclocondensation of **5** with hydrazine hydrate in boiling ethanol afforded the corresponding pyrazoline **6**. While, cyclocondensation with hydrazine hydrate in boiling acetic acid furnished the acetyl pyrazoline **7**. Also, when the chalcone **5** was allowed to react with phenyl hydrazine or thiosemicarbazide in alkaline medium, the 4,5-dihydropyrazole derivatives **8** and **9** were afforded, respectively. Structure of the pyrazoline derivatives **6–9** was estimated by careful studying of their spectral data and elemental analyses. Where, their ¹H NMR spectra revealed the signals of CH₂ protons of the pyrazoline ring at δ : 3.30–3.50 and 3.60–4.40 p.p.m. regions, while CH proton appeared at δ : 5.80–6.00 p.p.m. region. The reaction of chalcone with hydrazine derivatives took place according to the reported mechanism (Farag et al. 2015), where, cyclization of chalcone with substituted hydrazines for formation the pyrazoline takes place as follow: at first condensation of the free amino group of hydrazine derivative with carbonyl group of the chalcone followed by addition of the NH group of the hydrazine to the olefinic bond of the chalcone.

Moreover our interest was directed to study the reactivity of the quinazolin-2-aldehyde **3** with some different types of nitrogen nucleophiles for synthesizing newer biologically

active derivatives. Thus, reaction of aldehyde **3** with some aromatic amines and 4-aminoantipyrine afforded the corresponding Schiff's base derivatives **10a–c** in good yields. Treatment of **3** with phenylhydrazine or isonicotinichydrazide, furnished the novel hydrazone derivatives **11** and **12**, respectively (Scheme 4). ¹H NMR spectrum of **10c**, as representative to the products formed, displayed characteristic singlet at δ : 1.79 and 3.27 p.p.m. assigned to CH₃ and N–CH₃ protons, respectively. Finally, aiming to reach new biologically active compounds, some pyrane derivatives were synthesized. So, multi-component reaction of the aldehyde **3** with malononitrile and ethyl acetoacetate in the presence of piperidine afforded the pyrane derivative **13**. On the other hand, when ethyl acetoacetate was replaced by β -naphthol, the pyrane derivative **14** was obtained (Scheme 4). IR spectrum of the 4*H*-pyran-3-carboxylates **13** showed characteristic bands for NH₂ at ν : 3332, 3256, C \equiv N at ν : 2194 and C=O at ν : 1709, 1681 cm⁻¹. Its ¹H NMR spectrum revealed characteristic signals for ethyl protons at δ : 1.08 and 3.96 p.p.m. and singlet signal at δ : 2.07 for CH₃. A broad signal at δ : 6.99 (D₂O exchangeable) for NH₂ has appeared imbedded with the aromatic protons around δ : 7.41–8.16 p.p.m. region. Mass spectrum of **13** revealed molecular ion peak at m/z (M⁺) = 622.

Scheme 4 Syntheses of quinazolinone Schiff's bases and hydrazone derivatives **10–12** and pyrane derivatives **13** and **14**. Reagent and condition: (a) ArNH₂ or 4-aminoantipyrene, dioxane, reflux, 1–1.5 h; (b) 4-phenylhydrazine, dioxane, reflux, 0.5 h; (c) isonicotinichydrazide, dioxane, reflux, 0.75 h; (d) malononitrile, ethyl acetoacetate and piperidine (cat.), acetonitrile, stirred r.t. 3 h; (e) malononitrile, β -naphthol and piperidine (cat.), methanol, reflux 10 h



Biological activities

Target of the present work is the synthesis of different series of quinazolinone derivatives bearing the iodine atom at position-6, 3-trifluoromethyl phenyl at position-3 and various substituent at position-2 at the 4(3*H*)-quinazolinone moiety as a trial to acquire more secure and potent antitumor and antiviral agents. The antitumor and antiviral activities of the synthesized derivatives were carried out. The effect of substituent at position-2 of 4(3*H*)-quinazolinone moiety on the biological activities was studied. A comparative study to deduce a structure activity relationship (SAR) was pointed out and discussed.

Antitumor properties

Antitumor properties of the synthesized compounds in comparison with the standard drug, doxorubicin was evaluated by using sulforhodamine B colorimetric (SRB) assay (Skehan et al. 1990) for HepG2, MCF-7, and A549 cell lines. Most of the tested compounds were found to be potent against both cell lines HepG2 and MCF-7. IC₅₀ values ranging from 6.45 to 39.64 nM/mL for HepG2 from 11.82 to 52.29 nM/mL for MCF-7, while the IC₅₀ values of standard drug doxorubicin was 5.34 and 6.81 nM/mL for HepG2 and MCF-7, respectively. Also, the results showed that compound **4a** was found to be potent similar to the doxorubicin against HepG2 and MCF-7 cell lines (Table 1).

Certain features of the structures activity relationships of the prepared compounds were clearly highlighted; the substitution at position-2 was referred by X. From the obtained results, a clear difference in cytotoxicity was noted between the tested compounds depending on X group. The results demonstrated the following assumptions about the structural activity relationship (SAR).

The presence of thiosemicarbazone moiety in compounds **4a–f** showed the highest cytotoxic activity among

Table 1 The IC₅₀ values of the tested synthesized compounds against three human tumor cell lines

Compound No.	IC ₅₀ (nM/mL) of Cytotoxicity		
	HepG2	MCF-7	A549
4a	7.54	11.82	N.A.
4b	18.08	21.36	N.A.
4c	20.18	27.16	N.A.
4d	26.81	30.35	N.A.
4e	16.20	18.99	N.A.
4f	10.02	13.56	N.A.
5	39.64	N.A.	N.A.
6	22.13	31.53	N.A.
7	34.58	N.A.	N.A.
8	9.08	13.85	108.08
9	31.34	52.29	129.16
10a	N.A.	N.A.	N.A.
10b	N.A.	N.A.	N.A.
10c	N.A.	34.98	N.A.
11	35.02	36.89	N.A.
12	N.A.	N.A.	N.A.
13	N.A.	N.A.	N.A.
14	6.45	11.95	116.04
Doxorubicin	5.34	6.81	7.92

N.A. no activity

all the investigated compounds. Compounds **4a–f**, bearing *N*-substituted thiosemicarbazone moiety where *N*-substituted are **4a**; H, **4b**; methyl, **4c**; ethyl, **4d**; phenyl, **4e**; 4-fluorophenyl, **4f**; cyclohexyl. In general, incorporation *N*-substituted thiosemicarbazone moiety improves the cytotoxic activity. Thus, the unsubstituted thiosemicarbazone side chain moiety **4a** displayed strong effect on the cytotoxic activity. The latter compound is one of the highest cytotoxic compound among all the compounds investigated

in this study; where **4a** showed results near to the reference drug. Also, cyclohexyl moiety in **4f** showed good activity with 50% less potent effect than doxorubicin against HepG2 (IC₅₀ 10.02 nM) and MCF-7 (IC₅₀ 13.56 nM). Compound **4b** bearing methyl moiety showed activity equipotent to compound **4e** bearing 4-fluorophenyl moiety. The latter compounds showed about 30% less potent effect than doxorubicin, against HepG2. Moreover, the presence of phenyl moiety in **4d** displayed detrimental effect on the cytotoxic activity with lowest cytotoxic activity among this series. Compound **5**, which has α,β -unsaturated ketone side chain moiety ending with 4-methylphenyl moiety showed moderate activity (IC₅₀ 39.64 nM) against HepG2 cell line only. Regarding to compounds **6–9**, where X are *N*¹-substituted pyrazoline derivatives (*N*-substituted are **6**; hydrogen, **7**; COCH₃, **8**; phenyl, **9**; CSNH₂), it was noticed, generally, that treatment α,β -unsaturated ketone **5** with different hydrazines proved to improve the Antitumor properties. The presence of phenyl moiety in compound **8** showed good activity against HepG2 (IC₅₀ 9.08 nM) and MCF-7 cell lines (IC₅₀ 13.85 nM), but the unsubstituted pyrazoline derivative **6** exhibited moderate cytotoxic activity against HepG2 and MCF-7 cell lines. For compounds **10–12**, where X is *N*-substituted azomethine side chain moiety (*N*-substituted are: phenyl, **10a**; 3-CF₃-C₆H₄, **10b**; antipyrin-4-yl, **10c**; NHPPh, **11**; NHCO-pyridin-4-yl **12**);). It was noticed that the presence of antipyrin-4-yl moiety in compound **10c** showed moderate activity against MCF-7 cell line (IC₅₀ 34.98 nM) and no activity against the other tested cell lines. Introduction of NHPPh moiety in case of compound **11** showed moderate activities against HepG2 and MCF-7 cell lines. Replacement of NHPPh moiety by a pyridine amide or substituted pyrane moiety has a detrimental effect; where compounds **12** and **13** showed no activity against all of the tested cell lines. While increasing the size of the X substituent, as in case of structure **14**, revealed a good effect on cytotoxic activity against HepG2 and MCF-7, and moderate activity against A549.

Antiviral activity evaluation in vitro cell line

Most of the synthesized 4(3*H*)-quinazolinone derivatives were evaluated for their inhibitory effect on the replication of H₅N₁ virus, as well as toxicity evaluation in vitro cell lines. The toxicity properties of the synthesized compounds were tested in Madin Darby Canine kidney (MDCK) cells by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Mossman 1983). Evaluation of the antiviral activity of 4(3*H*)-quinazolinone derivatives in comparison with Zanamivir as a standard drug was determined according to Plaque reduction assay (Hayden et al. 1980), depicted in (Table 2). A plaque is a localized focus of virus-infected cells which under optimal

Table 2 The IC₅₀ values in Vero cell line and antiviral activity measured using Plaque reduction assay

Compd. No.	Toxicity evaluation in viro cell line (TC ₅₀ µg/µL)	Antiviral activity	
		Conc. µg/µL	% of Inhibition
4a	331	20	11
		40	21
4b	285.5	20	0
		40	3
4c	215	20	29
		40	47
4d	281	20	13
		40	13
5	83.5	20	28
		40	38
8	447	20	0
		40	0
10c	286	20	1
		40	3
12	145.6	20	8
		40	25
13	118.7	20	15
		40	15
Zanamivir		10	100

conditions originates from a single infectious virus particle. Counting of these foci for serial dilution of virus suspension is a highly quantitative method for assay of viral infectivity. Under these conditions, reduction in virus plaque counts provides a very sensitive mean for measuring antiviral activity of potential antiviral. The results of toxicity (TC₅₀) and the plaque reduction assay at two different concentrations 20, 40 (µg/mL) are summarized in (Table 2).

Compounds **4a–d**, **5**, **8**, **10c**, **12**, and **13** were evaluated for antiviral activity against influenza A (H₅N₁) virus in cell culture. From the results depicted in Table 2, it can be concluded, generally, that the majority of the tested compounds were of weak to moderate activity. Where, thiosemicarbazone **4c**, chalcone **5** and hydrazide **12** exhibited moderate curative activities, but compounds **4a**, **4d**, and **13** exhibited weak inhibitory. Both titled compounds **4b** and **10c** exhibited very weak inhibitory activities.

Conclusion

In general, it can be concluded that treatment of 4(3*H*)-quinazolinone-2-carboxaldehyde with *N*-substituted thiosemicarbazide derivatives improved the antitumor properties against the tested cell lines. Also, the presence of pyrazoline

moiety, obtained from reaction of α,β -unsaturated ketone with hydrazines, enhanced the antitumor activity depending on the type of the substituent on the pyrazoline moiety; while, incorporation benzo[*g*]chromene moiety showed good activity against HepG2 and MCF-7, and moderate activity against A549. From the obtained data regarding antiviral activity against influenza A (H_5N_1) virus in cell culture, it can be deduced that majority of the tested compounds were of moderate to weak activity. Where, the 4-(3*H*)-quinazolinone derivative **8c** bearing *N*-ethyl thiosemicarbazone side chain moiety showed 47% antiviral activity compared with Zanamivir drug.

Experimental part

All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus, (Shimadzu, Tokyo, Japan). Microanalyses were carried out by the Micro analytical Laboratory, National Research Centre, Cairo, Egypt. Infrared spectra (KBr-disc) were recorded using a Jasco FT/IR-300E spectrometer. 1H NMR and ^{13}C NMR spectra were measured in DMSO-*d*₆ using Varian Mercury 500 MHz and Varian Gemini 200 MHz with chemical shifts using TMS as standard solvent. Mass spectra were recorded on a GC/MS Finnigan SSQ 7000 spectrometer. Reactions were monitored by TLC on 0.25 mm Merck Silica gel sheets (60 GF 354) (4 × 2 cm) and the spots were detected with UV light.

Synthesis of 6-Iodo-2-methyl-3-(3-trifluoromethylphenyl)-3*H*-quinazolin-4-one (**2**)

Method A

A mixture of the benzoxazine **1** (0.01 mol) and trifluoromethylaniline (0.01 mol) in glacial acetic acid (30 ml) containing few amounts of freshly prepared fused sodium acetate was heated under reflux for 3 h, allowed to cool. The reaction mixture was poured onto crushed ice. The obtained solid product was filtered and crystallized from ethanol to give quinazolinone derivative **2**, yield 92%.

Method B

Benzoxazine **1** (0.01 mol) was fused with little excess amount of trifluoromethylaniline at ~150 °C in an oil-bath for 20 min. The solid product remained was triturated with acetic acid (20 ml) then poured onto crushed ice. The solid product obtained was filtered and crystallized from ethanol to give **2**, yield 85%. m.p. 220 °C; IR: ν/cm^{-1} : 1678 (C=O); 1H NMR: $\delta/p.p.m.$: 2.07 (s, 3H, CH₃), 7.44 (d, 1H, *J* = 8.40 Hz, Ar-H at C₈-H quinazoline), 7.70–7.93 (m, 3H, Ar-H), 7.97 (s, 1H, Ar-H), 8.21 (d, 1H, *J* = 8.40 Hz, Ar-H

at C₇-H quinazoline), 8.32 (s, 1H, Ar-H at C₅-H quinazoline); ^{13}C NMR: 23.3 (CH₃), 95.8, 121.0, 122.2, 124.0, 124.3, 126.1, 129.3, 131.0, 131.4, 136.1, 140.3, 142.5, 145.1, 154.8, 161.0 (C=O); Anal. Calcd for C₁₆H₁₀F₃IN₂O (430.16): C, 44.67; H, 2.34; N, 6.51; Found: C, 44.33; H, 2.15; N, 6.84%.

Synthesis of 6-iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-carbaldehyde (**3**)

2-Methyl-3(4*H*)-quinazolinone **2** (0.01 mol) was dissolved in hot dioxane (50 ml), selenium dioxide (0.02 mol) was added portion-wise while stirring. After complete addition, the reaction mixture was boiled with stirring for 6 h. The reaction mixture was then filtered off. The filtrate was poured onto crushed ice, the solid product obtained was filtered and crystallized from benzene to give **3** as beige crystals, Yield 65%; m.p. 250 °C; IR: ν/cm^{-1} : 1719, 1687 (C=O); 1H NMR: δ/ppm : 7.65–7.83 (m, 4H, Ar-H), 7.86 (s, 1H, Ar-H), 8.29 (d, 1H, Ar-H at C₇-H quinazoline), 8.45 (s, 1H, Ar-H at C₅-H quinazoline), 9.43 (s, 1H, CHO); ^{13}C NMR: 94.3, 121.4, 123.0, 124.1, 124.5, 126.8, 129.7, 131.8, 132.1, 133.2, 137.4, 143.1, 144.0, 160.1, 163.4, 192.1; Anal. Calcd for C₁₆H₈F₃IN₂O₂ (444.15): C, 43.27; H, 1.82; N, 6.31; Found: C, 43.43; H, 1.51; N, 6.54%.

Synthesis of thiosemicarbazone derivatives (**4a–f**)

A mixture of aldehyde **3** (0.01 mol) and the desired thiosemicarbazide derivative (namely, thiosemicarbazide; 4-methyl-, 4-ethyl-, 4-phenyl, 4-(4-fluorophenyl)-, 4-cyclohexylthiosemicarbazide) (0.012 mol) in methanol (25 mL) was stirred at room temperature for 1 h. The solid obtained was filtered and crystallized from ethanol to give the desired thiosemicarbazone derivatives **4a–f** as greenish yellow crystals.

2-((6-Iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)methylene)-hydrazinecarbothioamide (**4a**)

Yield 82%; m.p. 248 °C; IR: ν/cm^{-1} : 3420, 3127 (NH, NH₂), 1681 (C=O), 1597 (C=N); 1H NMR: $\delta/p.p.m.$: 6.37 (br, 2H, NH₂, D₂O-exchangeable), 7.55–7.59 (m, 2H, Ar-H), 7.76–7.86 (m, 4H, Ar-H+CH=N), 8.16 (d, 1H, *J* = 8.50 Hz, Ar-H at C₇-H quinazoline), 8.40 (s, 1H, Ar-H at C₅-H quinazoline), 11.71 (br, 1H, NH, D₂O-exchangeable); ^{13}C NMR: 94.4, 116.9, 124.1, 126.5, 128.3, 129.1, 129.5, 131.0, 132.2, 134.8, 135.7, 136.9, 150.9, 159.3, 163.4, 173.0, 177.1; MS (*m/z*: %): 518 (M⁺, 69.6), 402 (53.9), 287 (100), 146 (38.2); Anal. Calcd. for C₁₇H₁₁F₃I-N₅OS (517.27); Requires: C, 39.47; H, 2.14; N, 13.54; Found: C, 39.26; H, 1.97; N, 13.35%.

2-((6-Iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)methylene)-N-methylhydrazinecarbothioamide (**4b**)

Yield 85%; m.p. 226 °C; IR: ν/cm^{-1} : 3400, 3146 (2NH), 1685 (C=O) and 1594 (C=N); ^1H NMR: $\delta/\text{p.p.m.}$: 2.83 (d, 3H, $J = 6.80$ Hz, CH_3), 7.55 (br, 1H, NH, D_2O -exchangeable), 7.55–7.57 (m, 2H, Ar-H), 7.77–7.97 (m, 4H, Ar-H + CH=N), 8.16 (d, 1H, $J = 8.50$ Hz, Ar-H at $\text{C}_7\text{-H}$), 8.42 (s, 1H, Ar-H at $\text{C}_5\text{-H}$), 11.80 (br, 1H, NH, D_2O -exchangeable); ^{13}C NMR: 32.2, 94.4, 117.1, 124.3, 126.4, 128.1, 129.5, 129.3, 131.4, 132.8, 134.2, 135.9, 137.0, 150.1, 159.0, 163.8, 170.4, 173.1, 178.4; MS (m/z : %): 531 (M^+ , 17.9), 462 (100), 441 (8.24), 415 (15.59), 302 (18.5), 145 (34), 116 (42.1); Anal. Calcd for $\text{C}_{18}\text{H}_{13}\text{F}_3\text{IN}_5\text{OS}$ (531.29): C, 40.69; H, 2.47; N, 13.18; Found: C, 40.97; H, 2.24; N, 13.33%.

N-Ethyl-2-((6-iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)hmethylene)hydrazine carbothioamide (**4c**)

Yield 86%; m.p. 206 °C; IR: ν/cm^{-1} : 3354, 3129 (2NH) and 1687 (C=O); ^1H NMR: $\delta/\text{p.p.m.}$: 1.01 (t, 3H, $J = 6.80$ Hz, CH_3), 3.40 (q, 2H, $J = 6.80$ Hz, CH_2), 7.21 (t, 1H, $J = 6.20$ Hz, NH, D_2O -exchangeable), 7.56 (d, 2H, $J = 6.85$ Hz, Ar-H), 7.77–7.84 (m, 2H, Ar-H+CH=N), 7.93 (d, 1H, $J = 6.85$ Hz, Ar-H), 7.96 (s, 1H, Ar-H), 8.18 (d, 1H, $J = 8.50$ Hz, Ar-H at $\text{C}_7\text{-H}$ quinazoline), 8.37 (s, 1H, Ar-H at $\text{C}_5\text{-H}$ quinazoline), 11.75 (br, 1H, NH, D_2O -exchangeable); ^{13}C NMR: 14.4 (CH_3), 39.3 (CH_2), 94.4, 121.0, 123.7, 124.5, 124.6, 126.9, 129.4, 131.3, 132.7, 133.5, 137.1, 143.4, 144.8, 160.6, 163.4, 164.7; 177.1; MS (m/z : %): 545 (M^+ , 11.9), 461 (48.7), 441 (24.8), 360 (18.5), 302 (18.5), 233 (21.2), 145 (32.8), 60 (100); Anal. Calcd for $\text{C}_{19}\text{H}_{15}\text{F}_3\text{IN}_5\text{OS}$ (545.32): C, 41.85; H, 2.77; N, 12.84; Found: C, 41.64; H, 2.54; N, 12.67%.

2-((6-Iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)methylene)-N-phenylhydrazinecarbothioamide (**4d**)

Yield 86%; m.p. 236 °C; IR: ν/cm^{-1} : 3437, 3127 (2NH), 1688 (C=O) and 1516 (C=N); ^1H NMR: $\delta/\text{p.p.m.}$: 7.19 (t, 1H, $J = 7.63$ Hz, Ph-H), 7.34 (t, 2H, $J = 7.65$ Hz, Ph-H), 7.42 (d, 2H, $J = 7.60$ Hz, Ph-H), 7.59–7.81 (m, 5H, Ar-H + CH=N), 7.98 (s, 1H, Ar-H), 8.18 (d, 1H, $J = 8.40$ Hz, Ar-H at $\text{C}_7\text{-H}$ quinazoline), 8.39 (s, 1H, Ar-H at $\text{C}_5\text{-H}$ quinazoline), 9.44 (br, 1H, NH, D_2O -exchangeable), 12.01 (br, 1H, NH, D_2O -exchangeable); ^{13}C NMR: 94.4, 120.1, 123.0, 124.4, 124.8, 125.5 (2C), 127.1 (2C), 128.4, 128.2, 129.2, 131.1, 131.7, 133.5, 136.4, 139.1, 142.1, 144.5, 154.9, 160.4, 163.4, 176.4; MS (m/z : %): 593 (M^+ , 3.1),

590 (3.8), 558 (4.5), 500 (8.7), 441 (14.6), 288 (18.5), 245 (24.5), 145 (47.6), 93 (100); Anal. Calcd for $\text{C}_{23}\text{H}_{15}\text{F}_3\text{IN}_5\text{OS}$ (593.36): C, 46.56; H, 2.55; N, 11.80; Found: C, 46.35; H, 2.34; N, 11.94%.

N-(4-Fluorophenyl)-2-((6-iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)methylene)hydrazinecarbothioamide (**4e**)

Yield 88%; m.p. 234 °C; IR: ν/cm^{-1} : 3426, 3135 (2NH), 1688 (C=O) and 1597 (C=N), 1597 (C=N); ^1H NMR: $\delta/\text{p.p.m.}$: 7.18–7.97 (m, 10H, Ar-H+CH=N), 8.00 (d, 1H, Ar-H at $\text{C}_7\text{-H}$ quinazoline), 8.39 (s, 1H, Ar-H at $\text{C}_5\text{-H}$ quinazoline), 8.80 (br, 1H, NH, D_2O -exchangeable), 12.12 (br, 1H, NH, D_2O -exchangeable); ^{13}C NMR: 94.1, 120.3, 123.4, 124.2, 124.4, 125.8 (2C), 126.1, 127.1, 128.4, 128.2, 129.2, 132.0, 131.7, 133.5, 136.4, 139.1, 142.1, 144.2, 155.3, 161.2, 163.7, 177.1; MS (m/z : %): 611 (M^+ , 4.7), 440 (45.3), 359 (14.8), 287 (18.8), 216 (31.3), 144 (60.9), 111 (100), 74 (89.1); Anal. Calcd for $\text{C}_{23}\text{H}_{14}\text{F}_4\text{IN}_5\text{OS}$ (611.35): C, 45.19; H, 2.31; N, 11.46; Found: C, 45.34; H, 2.45; N, 11.57%.

N-Cyclohexyl-2-((6-iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)methylene)hydrazinecarbothioamide (**4f**)

Yield 92%; m.p. 242 °C; IR: ν/cm^{-1} : 3354, 3138 (2NH), 1685 (C=O); ^1H NMR: $\delta/\text{p.p.m.}$: 1.05–1.19 (m, 4H, 2 CH_2), 1.55 (q, 2H, CH_2), 1.73 (m, 4H, 2 CH_2), 2.43 (q, 1H, CH cyclohexane), 6.72 (d, 1H, $J = 6.20$ Hz, NH, D_2O -exchangeable), 7.56–8.37 (m, 8H, Ar-H+CH=N), 11.74 (br, 1H, NH, D_2O -exchangeable); ^{13}C NMR: 25.4 (2C), 25.7, 32.0 (2C), 54.3, 94.3, 121.4, 123.0, 124.1, 124.5, 126.8, 129.7, 131.8, 132.1, 133.2, 137.4, 143.1, 144.0, 160.1, 163.4, 164.1; 176.1; MS (m/z : %): 598.9 (9.2, M^+), 444.9 (10.8%), 184 (6.1), 145 (20.9), 98.10 (62.6), 83.05 (37.1), 55 (100); Anal. Calcd for $\text{C}_{23}\text{H}_{21}\text{F}_3\text{IN}_5\text{OS}$ (599.41): C, 46.09; H, 3.53; N, 11.68; Found: C, 46.21; H, 3.46; N, 11.78%.

Synthesis of 6-iodo-2-(3-oxo-3-*p*-tolylprop-1-enyl)-3-(3-(trifluoromethyl)phenyl)-quinazolin-4(3*H*)-one (**5**)

A mixture of the aldehyde derivative **3** (0.01 mol), 4-methyl acetophenone (0.01 mol) and NaOH (0.01 mol) in aqueous methanol (20 mL) was heated under reflux for 1 h, left to cool. The solid product obtained was filtered off and crystallized from methanol to give **5** as yellow crystals. Yield 81%; m.p. 200 °C; IR: ν/cm^{-1} : 1680, 1609 (2C=O), 1563 (C=C); ^1H NMR: $\delta/\text{p.p.m.}$: 2.34 (s, 3H, CH_3), 6.67 (d, 1H, CH=CH), 7.32–8.40 (m, 10H, Ar-H+CH=CH), 8.21 (d, 1H, $J = 8.40$ Hz, Ar-H), 8.40 (s, 1H, Ar-H); ^{13}C NMR:

21.1, 97.0, 116.3, 116.7, 121.7, 123.3, 124.8, 124.1, 128.9, 129.2, 129.6, 130.1, 130.9, 131.1, 131.3, 132.4, 137.6, 139.6, 142.2, 143.9, 148.3, 156.5, 165.1, 166.3, 168.6; MS (*m/z*: %): 560 (M^+ , 37.4), 468.9 (7.2), 440.9 (87.9), 215.9 (13.9), 145 (83.9), 119.1 (100), 91.1 (99.4); Anal. Calcd for $C_{25}H_{16}F_3IN_2O_2$ (560.31): C, 53.59; H, 2.88; N, 5.00; Found: C, 53.33; H, 2.67; N, 5.17%.

Synthesis of 6-iodo-2-(3-*p*-tolyl-4,5-dihydro-1*H*-pyrazol-5-yl)-3-(3-(trifluoromethyl)phenyl) quinazolin-4(3*H*)-one (6)

To a solution of the chalcone **5** (0.01 mol) in ethanol (60 ml), hydrazine hydrate (0.012 mol) was added. The reaction mixture was heated under reflux for 4 h. After cooling the separated solid was filtered and washed with a little ethanol and recrystallized from ethanol to give compound **6**. Yield 73%; m.p. > 300 °C; IR: ν/cm^{-1} : 1701 (C=O); 1H NMR: $\delta/p.p.m.$: 2.43 (s, 3H, CH_3), 3.33 (m, 1H, pyrazoline-H) 3.76 (m, 1H, pyrazoline-H), 5.66 (m, 1H, pyrazoline-H), 7.24–8.46 (m, 11H, Ar-H), 12.56 (s, 1H, NH); ^{13}C NMR: 22.3, 86.9, 96.4, 121.6, 124.1, 124.2, 128.8, 129.3, 129.9, 130.2, 130.8, 131.2, 131.8, 132.6, 137.5, 139.1, 142.7, 143.6, 148.4, 155.1, 163.4, 164.3, 165.4, 166.3, 168.8; MS (*m/z*: %): 574 (M^+ ; 55.5), 550 (65.4), 494 (64.1), 433 (54.3), 389 (47.6), 271 (61.3), 259 (60.4), 95 (58.1), 72 (57.4), 69 (100); Anal. Calcd for $C_{25}H_{18}F_3IN_4O$ (574.34): C, 52.28; H, 3.16; N, 9.76; Found: C, 52.45; H, 3.01; N, 9.87%.

Synthesis of 2-(1-acetyl-3-*p*-tolyl-4,5-dihydro-1*H*-pyrazol-5-yl)-6-(trifluoromethyl)-3-(3-(trifluoromethyl)phenyl)quinazolin-4(3*H*)-one (7)

A mixture of chalcone derivative **5** (0.01 mol) and hydrazine hydrate (0.012 mol) in 30 ml glacial acetic acid was heated under reflux for 8 h., after cooling to ambient temperature the reaction mixture was poured into ice cold water (50 ml). The formed precipitate was filtered off, dried, and crystallized from ethanol/ DMF (3:1) to give compound **7**. Yield 68%; m.p. > 300 °C; IR: ν/cm^{-1} : 1701, 1630 (2 C=O); 1H NMR: $\delta/p.p.m.$: 2.31 (s, 3H, $COCH_3$), 2.43 (s, 3H, CH_3), 3.23 (m, 1H, pyrazoline-H), 3.68 (m, 1H, pyrazoline-H), 5.92 (m, 1H, pyrazoline-H), 7.2–8.5 (m, 11H, Ar-H); ^{13}C NMR: 21.4, 24.3, 87.5, 96.9, 121.7, 122.0, 124.3, 128.8, 129.2, 129.7, 130.3, 130.8, 131.3, 131.6, 132.5, 137.6, 139.5, 142.2, 143.9, 148.5, 150.1, 163.2, 165.7, 166.4, 168.9; MS (*m/z*: %): 616.1 (M^+ , 97.2), 577.1 (47.6), 524.1 (65.9), 483.1 (55.1), 414.1 (100), 145.1 (6.8), 74.8 (89.1); Anal. Calcd for $C_{27}H_{20}F_3IN_4O_2$ (616.37): C, 52.61; H, 3.27; N, 9.09; Found: C, 52.36; H, 3.35; N, 9.18%.

Synthesis of 6-iodo-2-(1-phenyl-5-*p*-tolyl-4,5-dihydro-1*H*-pyrazol-3-yl)-3-(3-(trifluoromethyl)-phenyl) quinazolin-4(3*H*)-one (8)

To a mixture of chalcone **5** (0.01 mol) and phenylhydrazine (0.01 mol) in ethanol (50 mL), NaOH (20 mmol) in 5 mL of water was added. The reaction mixture was heated under reflux for 6 h. The completion of the reaction was monitored by TLC. The reaction mixture was allowed to cool down to room temperature and poured into ice cooled water with constant stirring. The resulting precipitate was filtered, washed with water, dried and recrystallized from ethanol to produce **8** as yellow crystals, yield 59%, m.p. 280 °C; IR: ν/cm^{-1} : 1676 (C=O); 1H NMR: $\delta/p.p.m.$: 2.41 (s, 3H, CH_3), 3.52 (m, 1H, pyrazoline-H), 4.38 (m, 1H, pyrazoline-H), 6.00 (m, 1H, pyrazoline-H), 6.80–8.50 (m, 16H, Ar-H); ^{13}C NMR: 21.2, 87.4, 96.7, 116.3 (2C), 121.3, 121.5, 121.8, 124.4, 128.7 (3C), 129.2, 129.5 (3C), 130.3, 130.9, 131.4, 131.6, 132.5, 137.9, 139.6, 142.3, 143.9, 148.5, 150.1, 163.2, 165.6, 166.3, 168.8; MS (*m/z*: %): 650 (6.5), 648 (65.4), 521 (14.1), 443 (54.3), 389 (11.3), 235 (37.4), 145 (30), 119 (100), 91 (77.1); Anal. Calcd for $C_{31}H_{22}F_3IN_4O$ (650.43): C, 57.24; H, 3.41; N, 8.61; Found: C, 57.13; H, 3.21; N, 8.52%.

Synthesis of 3-(6-iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)-5-*p*-tolyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (9)

To a mixture of chalcone **5** (0.01 mol) and thiosemicarbazide (0.01 mol) in ethanol (50 mL), NaOH (20 mmol) in 5 mL of water was added and heated under reflux for 10 h. The reaction mixture was poured onto acidified crushed ice. The resulting solid was filtered, dried and recrystallized from ethanol. Yield 46%; m.p. > 300 °C; IR: ν/cm^{-1} : 3291 (NH_2), 1676 (C=O); 1H NMR: $\delta/p.p.m.$: 2.34 (s, 3H, CH_3), 3.42 (m, 1H, pyrazoline-H), 3.88 (m, 1H, pyrazoline-H), 5.62 (m, 1H, pyrazoline-H), 7.2–8.5 (m, 13H, Ar-H+ NH_2); ^{13}C NMR: 21.2, 86.9, 96.4, 121.6, 121.7, 124.2, 124.6, 128.7, 129.3, 129.8, 130.2, 130.8, 131.2, 131.7, 132.6, 137.4, 139.1, 142.6, 143.5, 148.4, 155.1, 163.4, 165.4, 166.3, 168.8, 176.1; Anal. Calcd for $C_{26}H_{19}F_3IN_5OS$ (633.43): C, 49.30; H, 3.02; N, 11.06; Found: C, 49.43; H, 3.14; N, 11.01%.

Synthesis of Schiff's bases and hydrazone derivatives (10–13)

A mixture of 4(3*H*)-quinazolinone-2-carboxaldehyde **3** (0.01 mol) and amino compound (0.01 mol) in dioxane (30 mL) was heated under reflux for appreciate time (0.5–1.5 h). Reaction was monitored by TLC. The reaction mixture was concentrated under vacuum and after cool the

ethanol was added. The solid product was recrystallized from ethanol to give the final products.

6-Iodo-2-((phenylimino)methyl)-3-(3-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (10a)

Yield 73%; m.p. 266 °C; IR: ν/cm^{-1} : 1680 (C=O), 1593 (C=N); $^1\text{H NMR}$: $\delta/\text{p.p.m.}$: 6.96 (s, 1H, Ar-H), 7.10–7.82 (m, 8H, Ar-H+CH=N), 8.02 (t, 2H, $J = 8.40$ Hz, Ar-H), 8.14 (d, 1H, $J = 8.40$ Hz, Ar-H), 8.34 (s, 1H, Ar-H); $^{13}\text{C NMR}$: 97.1, 116.1, 116.6, 120.6, 121.4, 121.6, 123.8, 124.0, 124.1, 126.4, 128.6, 129.6, 130.0, 132.7, 139.5, 146.5, 147.4, 153.5, 156.2, 164.3, 166.5, 167.3; Anal. Calcd for $\text{C}_{22}\text{H}_{13}\text{F}_3\text{IN}_3\text{O}$ (519.26): C, 50.89; H, 2.52; N, 8.09; Found: C, 50.62; H, 2.33; N, 8.34%.

6-Iodo-3-(3-(trifluoromethyl)phenyl)-2-((3-(trifluoromethyl)phenylimino)methyl)-quinazolin-4(3H)-one (10b)

Yield 73%, m.p. > 300 °C; IR: ν/cm^{-1} : 1680 (C=O), 1599 (C=N); $^1\text{H NMR}$: $\delta/\text{p.p.m.}$: 6.94 (s, 1H, Ar-H), 7.18 (d, 1H, $J = 8.40$ Hz, Ar-H), 7.71–7.84 (m, 6H, Ar-H+CH=N), 7.98 (t, 2H, $J = 8.40$ Hz, Ar-H), 8.12 (d, 1H, $J = 8.40$ Hz, Ar-H), 8.32 (s, 1H, Ar-H); $^{13}\text{C NMR}$: 100.2, 116.4, 120.8, 121.3, 121.5, 122.4, 123.9, 124.0, 124.1, 124.2, 126.4, 128.5, 129.4, 129.7, 131.3, 132.4, 139.5, 146.7, 147.4, 148.5, 164.5, 166.6, 173.0; MS (m/z : %): 587 (M^+ ; 26.8); 416 (92.7), 289 (31.7), 216 (48.8), 145 (95.1), 75 (100); Anal. Calcd for $\text{C}_{23}\text{H}_{12}\text{F}_6\text{IN}_3\text{O}$ (587.26): C, 47.04; H, 2.06; N, 7.16; Found: C, 47.34; H, 1.87; N, 7.01%.

2-((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylimino)methyl)-6-iodo-3-(3-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (10c)

Yield 80%; m.p. 262 °C; IR: ν/cm^{-1} : 1680, 1651 (2C=O), 1591 (C=N); $^1\text{H NMR}$: $\delta/\text{p.p.m.}$: 1.79 (s, 3H, CH_3), 3.27 (s, 3H, N- CH_3), 7.25 (d, 2H, $J = 7.65$ Hz, Ph-H), 7.36 (t, 1H, $J = 7.65$ Hz, Ph-H), 7.47 (t, 2H, $J = 7.65$ Hz, Ph-H), 7.57 (d, 1H, $J = 8.40$ Hz, Ar-H at C_8 -H quinazoline), 7.70–7.72 (m, 2H, Ar-H), 7.79 (d, 1H, $J = 6.85$ Hz, Ar-H), 7.90 (s, 1H, Ar-H), 8.21 (d, 1H, $J = 8.40$ Hz, Ar-H at C_7 -H quinazoline), 8.37 (s, 1H, Ar-H at C_5 -H quinazoline), 9.14 (s, 1H, CH=N); $^{13}\text{C NMR}$: 19.0 (CH_3), 35.0 (N- CH_3), 93.0, 114.2, 114.3, 122.7, 124.1, 124.6, 126.0, 126.1, 127.8, 128.9, 129.5, 129.9, 131.0, 132.9, 133.5, 134.8, 136.5, 143.3, 146.7, 146.9, 147.0, 150.6, 152.0, 157.8, 160.8; MS (m/z : %): 629 (M^+ ; 2.2), 628 (M-1; 7.0), 202 (6.8), 145 (2.1), 56 (100); Anal. Calcd for $\text{C}_{27}\text{H}_{19}\text{F}_3\text{IN}_5\text{O}_2$ (629.37): C, 51.53; H, 3.04; N, 11.13; Found: C, 51.21; H, 2.81; N, 10.89%.

6-Iodo-2-((2-phenylhydrazono)methyl)-3-(3-(trifluoromethyl)phenyl)quinazolin-4(3H)-one (11)

Yield 72%; m.p. 260 °C; IR: ν/cm^{-1} : 3432 (NH), 1681 (C=O), 1599 (C=N); $^1\text{H NMR}$: $\delta/\text{p.p.m.}$: 6.93–7.98 (m, 12H, ArH), 8.23 (s, 1H, CH=N), 8.46 (br, 1H, NH); $^{13}\text{C NMR}$: 94.5, 116.5, 122.5, 123.7, 124.2, 126.3, 128.1, 129.5, 129.7, 131.1, 131.3, 132.2, 134.6, 135.3, 136.7, 139.4, 146.3, 146.1, 152.3, 159.2, 163.1, 170.3; MS (m/z : %): 534 (M^+ ; 65.5), 533 (78.9), 429 (42.3), 360 (29.6), 302 (31.0), 145 (30.3), 77 (100); Anal. Calcd for $\text{C}_{22}\text{H}_{14}\text{F}_3\text{IN}_4\text{O}$ (534.27): C, 49.46; H, 2.64; N, 10.49; Found: C, 49.26; H, 2.34; N, 10.63%.

N'-((6-Iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)methylene)isonicotinohydrazide (12)

Yield 75%; m.p. 150 °C; IR: ν/cm^{-1} : 3211 (NH), 1686 (C=O), 1590 (C=N); $^1\text{H NMR}$: $\delta/\text{p.p.m.}$: 7.55–8.45 (m, 11H, Ar-H), 8.73 (br, 1H, NH), 9.42 (s, 1H, CH=N); $^{13}\text{C NMR}$: 94.4, 116.9, 123.0, 123.9, 124.1, 126.5, 128.3, 129.1, 129.5, 131.0, 132.2, 134.8, 135.7, 136.9, 139.8, 146.4, 151.4, 150.9, 159.3, 163.4, 170.3, 173.0; Anal. Calcd for $\text{C}_{22}\text{H}_{13}\text{F}_3\text{IN}_5\text{O}_2$ (563.27) C, 46.91; H, 2.33; N, 12.43; Found: C, 47.14; H, 2.21; N, 12.12%.

Synthesis of ethyl 6-amino-5-cyano-4-(6-iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)-2-methyl-4H-pyran-3-carboxylate (13)

A mixture of the aldehyde derivative **3** (0.01 mol), malononitrile (0.01 mol), ethyl acetoacetate (0.01 mol) and few drops of piperidine in acetonitrile was stirred at room temperature for 3 h. The solid product obtained was filtered off and crystallized from ethanol to give **13**. White crystals yield 70%, m.p. 195 °C; IR: ν/cm^{-1} : 3332, 3256 (NH_2), 2194 ($\text{C}\equiv\text{N}$) and 1709, 1681 (C=O); $^1\text{H NMR}$: $\delta/\text{p.p.m.}$: 1.08 (t, 3H, $J = 7.2$ Hz, CH_3), 2.07 (s, 3H, CH_3), 3.96 (q, 2H, $J = 7.2$ Hz, CH_2), 6.99 (br, 2H, NH_2 , D_2O -exchangeable), 7.41–8.16 (m, 8H, Ar-H+CH-pyrane); $^{13}\text{C NMR}$: 13.8, 18.4, 23.1, 57.4, 63.4, 94.7, 104.4, 118.3, 121.4, 122.4, 124.4 (2C), 125.9, 129.2, 130.2, 133.4, 137.2, 139.2, 143.3, 145.4, 157.5, 159.8, 161.6, 163.4, 167.2; MS (m/z : %): 622 (37.5), 575 (55.4), 548 (35.7), 504 (37.5), 428 (19.6), 351 (23.2), 288 (21.4), 207 (46.4), 161 (19.1), 145 (100), 127 (80.4), 75 (99.1); Anal. Calcd for $\text{C}_{25}\text{H}_{18}\text{F}_3\text{IN}_4\text{O}_4$ (622.33): C, 48.25; H, 2.92; N, 9.00; Found: C, 48.33; H, 3.02; N, 9.17%.

Synthesis of 2-amino-4-(6-iodo-4-oxo-3-(3-(trifluoromethyl)phenyl)-3,4-dihydroquinazolin-2-yl)-4*H*-benzo[*g*]chromene-3-carbonitrile (**14**)

A mixture of the aldehyde derivative **3** (0.01 mol), malonitrile (0.01 mol), and β -naphthol (0.01 mol) and few drops of piperidine in methanol was heated under reflux for 10 h, then the reaction after cool. The solid product obtained was recrystallized from ethanol to give **14**, yield 50%; m.p. 230 °C; IR: ν/cm^{-1} : 3445, 3417 (NH_2), 2222 ($\text{C}\equiv\text{N}$) and 1658 ($\text{C}=\text{O}$); ^1H NMR: $\delta/\text{p.p.m.}$: 6.11 (br, 2H, NH_2 , D_2O -exchangeable), 6.75 (m, 1H, Ar-H), 7.01 (m, 1H, Ar-H), 7.13 (m, 1H, Ar-H), 7.22–7.30 (m, 3H, Ar-H), 7.38 (m, 1H, Ar-H), 7.57 (d, 1H, $J = 8.40$ Hz, Ar-H), 7.86 (m, 1H, Ar-H), 8.19 (m, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 8.42 (m, 1H, Ar-H), 8.60 (m, 1H, Ar-H), 9.17 (s, 1H, CH-pyrane); ^{13}C NMR: 26.4, 59.9, 94.7, 107.7, 118.7, 121.4, 122.1, 123.9, 124.1, 124.6 (2C), 125.8 (2C), 126.9, 127.1, 129.0 (2C), 131.2 (2C), 131.4, 132.7, 136.2, 139.7, 142.3, 145.8, 155.1, 164.3, 160.6, 177.1; Anal. Calcd for $\text{C}_{29}\text{H}_{16}\text{F}_3\text{IN}_4\text{O}_2$ (636. 36): C, 54.73; H, 2.53; N, 8.80; Found: C, 54.47; H, 2.42; N, 8.63%.

Biological activity, materials, and methods

Chemicals

Fetal bovine serum (FBS) and L-glutamine were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and Sulfo-Rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma–Aldrich chemical Co. (St. Louis, MO, USA).

Cell lines and culturing

Anticancer activity screening for the tested compounds utilizing three different human cancer cell lines including, liver HepG2, breast MCF-7 and lung A549 cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The cancer cells were maintained in DMEM supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/mL) and streptomycin (100 $\mu\text{g}/\text{mL}$) at 37 °C in humidified atmosphere containing 5% CO_2 . Cells at a concentration of 0.50×10^6 were grown in a 25 cm^2 flask in 5 ml of complete culture medium.

In vitro cytotoxicity assay

The cytotoxicity activity was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the previous reported standard procedure (Skehan et al. 1990). Cells were inoculated in 96-well micro-titer plate (10^4 cells/well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO at 1 mg/mL immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentration of the tested compounds and doxorubicin were added to the cells. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2 . After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. 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. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1.

MTT cytotoxicity assay (TC_{50})

Samples were diluted with DMEM. Stock solutions of the test compounds were prepared in 10% DMSO in ddH_2O . The cytotoxic activity of the extracts were tested in Madin Darby Canine kidney (MDCK) cells by using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method (Mossman 1983). Briefly, the cells were seeded in 96 well-plates (100 $\mu\text{L}/\text{well}$ at a density of 3×10^5 cells/mL) and incubated for 24 h at 37 °C in 5% CO_2 . After 24 h, cells were treated with various concentrations of the tested compounds in triplicates. After further 24 h, the supernatant was discarded and cell monolayers were washed with sterile phosphate buffer saline (PBS) 3 times and MTT solution (20 μL of 5 mg/mL stock solution) was added to each well and incubated at 37 °C for 4 h followed by medium aspiration. In each well, the formed formazan crystals were dissolved with 200 μL of acidified isopropanol (0.04 M HCl in absolute isopropanol = 0.073 ml HCl in 50 ml isopropanol). Absorbance of formazan solutions were measured at λ_{max} 540 with 620 nm as a reference wavelength using a multi-well plate reader. The plot of % cytotoxicity vs. sample concentration was used to calculate the concentration which exhibited 50% cytotoxicity (TC_{50}).

Plaque reduction assay

Assay was carried out according to the method of Hayden et al. (Hayden et al. 1980) in a six well plate where MDCK cells (10^5 cells/mL) were cultivated for 24 h at 37 °C. A/chicken/M7217B/1/2013 (H_5N_1) virus was diluted to give 10^5 PFU/well and mixed with the safe concentration of the tested compounds, and incubated for 1 h at 37 °C before being added to the cells. Growth medium was removed from the cell culture plates and virus-Cpd or virus-extract and Virus-Zanamivir mixtures were inoculated (100 μ L / well), After 1 h contact time for virus adsorption, 3 ml of DMEM supplemented with 2% agarose was added onto the cell monolayer, plates were left to solidify and incubated at 37 °C till formation of viral plaques (3 to 4 days). Formalin (10%) was added for 2 h then plates were stained with 0.1% crystal violet in distilled water. Control wells were included where untreated virus was incubated with MDCK cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following: % inhibition = viral count (untreated)—viral count (treated)/viral count (untreated) \times 100

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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