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A Series of 2,4(1*H*,3*H*)-Quinazolinedione Derivatives: Synthesis and Biological Evaluation as Potential Anticancer Agents

Hulya Akgun[a,*], Demet Us Yilmaz[a], Rengul Cetin Atalay[b], and Damla Gozen[b]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Yeditepe University, 34755, Kayisdagi, Istanbul, Turkey*

[b]*Department of Molecular Biology and Genetics, BilGen, Genetics and Biotechnology Research Center, Faculty of Science, Bilkent University, 06800, Bilkent, Ankara, Turkey*

Hulya Akgun

Abstract: A series of 6,7-disubstituted-3-{2-[4-(substituted)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H,3H*)-dione derivatives (**7-34**) were synthesized and their structures were elucidated on the basis of analytical and spectral (UV, IR, ^{1}H-NMR, ^{13}C-NMR and MS) data. These synthesized compounds were evaluated for their *in vitro* cytotoxicities against a panel of three human cancer cell lines. According to the cytotoxicity screening results, 3-{2-[4-(4-chlorobenzyl)piperazin-1-yl]-2-oxoethyl} quinazoline-2,4(1*H*,3*H*)-dione (**7**) presented the highest activity against HUH-7, MCF-7 and HCT-116 cell line with the IC_{50} values of 2.5, 6.8 and 4.9 μM, respectively.

Keywords: Quinazoline, quinazoline-2,4(1*H,3H*)-dione, piperazine, cytotoxicity, anticancer, sulforhodamine B method.

1. INTRODUCTION

Cancer is one of the potentially fatal diseases, which is characterized by uncontrolled division of a group of cells leading to metastasis and invasion to adjacent tissues. Although many chemotherapeutic agents are in clinical use, synthesizing new anticancer agents with less side effect and increased selectivity is still ongoing area of interest in medicinal chemistry. Since the discovery of gefitinib (Fig. **1**), quinazolines has attracted considerable attention in the design of new chemotherapeutic agents. Some 4-anilinoquinazolines (erlotinib, vandetanib, lapatinib, afatinib and trimetrexate) and 4-quinazolinone derivative (raltitrexed) (Fig. **1**) have been introduced into clinical use for the treatment of several types of cancer. It was reported that, quinazolines which act as adenosine triphosphate (ATP)-mimic compounds show their tyrosine kinase enzyme inhibition activities by occupying the ATP-binding pocket with high affinity [1-4]. Quinazolines may also exhibit their anticancer activity by both p53 modulation and thyroid-stimulating hormone receptor (TSHR) agonistic activity [5, 6].

Various literatures report numerous 2,4(1*H*,3*H*)-quinazolinedione analogues showing a wide variety of biological activities such as anticancer [7-20], antimicrobial [21-37], antihypertensive [38-40], anticonvulsant [41-49], anti-inflammatory [50], 5-hydroxytryptamine 3 (5-HT_3) receptor antagonist [51], phosphodiesterase (PDE) 4 inhibition [52,53], calcium-independent phosphodiesterase enzyme inhibition (CaIPDE) [54], cyclin-dependent kinase 5 (CDK5) inhibition [55], 5-HT_{3A} receptor antagonist [56], antioxidant [57] and antiplatelet [58]. Some 3,6,7-trisubstituted-2,4-quinazolinedione derivatives were recently submitted to NCI antitumor screening program. Four of them (**1**, Fig. **2**) significantly inhibited growth of 60 human tumor cell *in vitro* with IC_{50} values ranging from 0.4 to 0.8 μM [7]. 3-Substituted-2,4-quinazolinedione derivative (**2**, Fig. **2**) was patented for its cytotoxicity with IC_{50} value below than 10 nM againts human ovarian cancer (SKOV3) cell line [8].

On the other hand, a comprehensive literature study shows that piperazines are a significant class of heterocyclic compound with their various biological properties (Fig. **3**), especially potential antitumour activity. Tahmatzopoulos *et al.* reported that the doxazosin and terazosin, α_1-adrenoceptor antagonists, could generate apoptosis in benign and malignant prostate cells, also decrease tumor vascularity in prostate tumors and suppress prostate tumorigenic growth *in vivo* [59].

In the light of these observations, with the aim of discovering new anticancer agents, a series of 6,7-disubstituted-3-{2-[4-(substituted)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H,3H*)-dione derivatives were synthesized (Fig. **4**) and their *in vitro* cytotoxicities were evaluated against three human cancer cell lines, hepatoma (HUH-7), breast cancer (MCF-7) and colorectal cancer (HCT-116) by using Sulforhodamine B test.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The synthetic routes for 3-substituted-2,4(1*H*,3*H*)-quinazolinedione derivatives are summarized in Scheme (**1**).

Synthesis of 2,4(1*H*,3*H*)-quinazolinedione ring derivatives started with the nucleophilic addition reaction of the 2-aminobenzoic acid derivatives to ethyl isocyanatoacetate

*Address correspondence to this author at the Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey; Tel/ Fax: +90 532 703 8399, +90 216 578 0068; E-mail: hakgun@yeditepe.edu.tr

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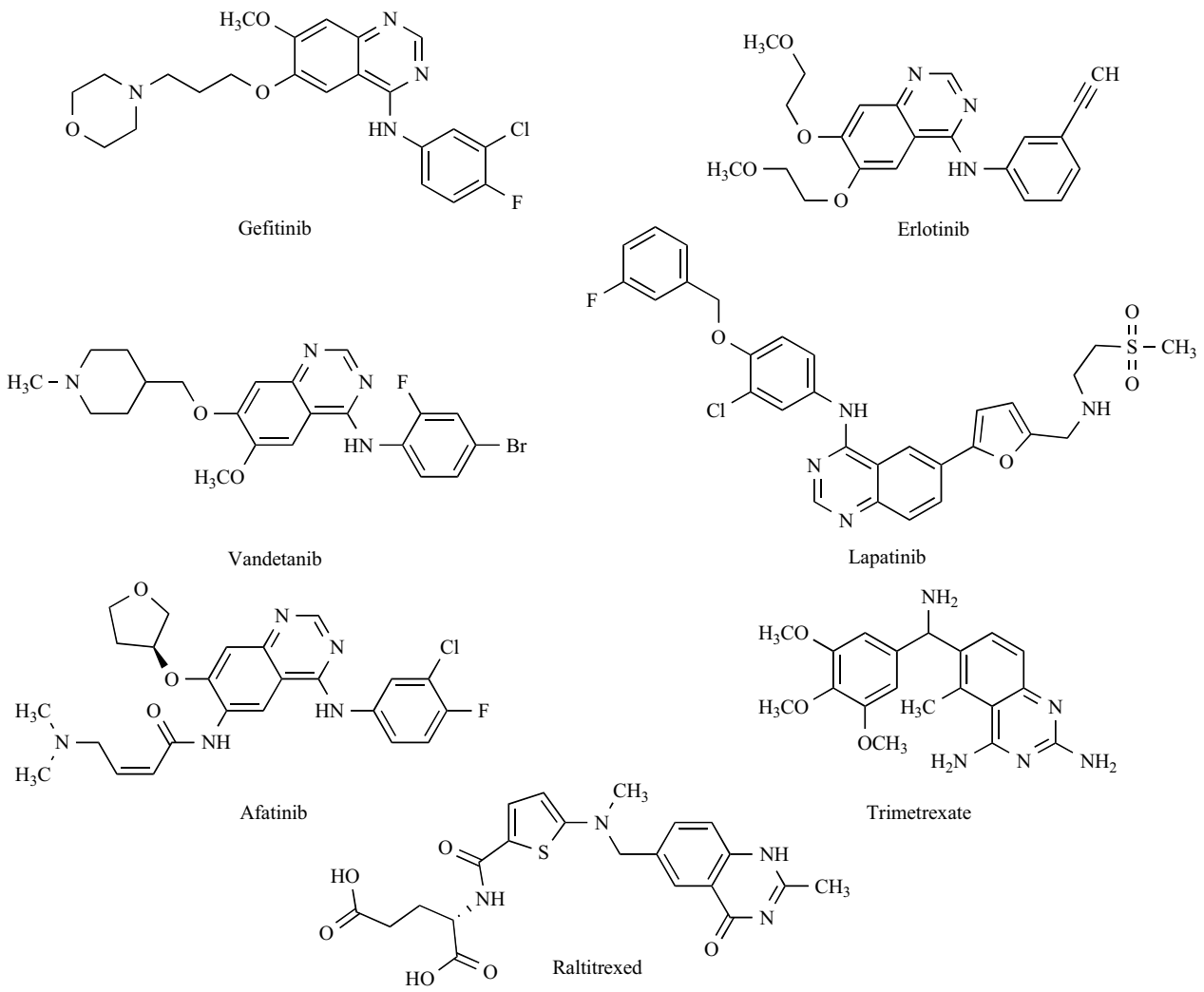
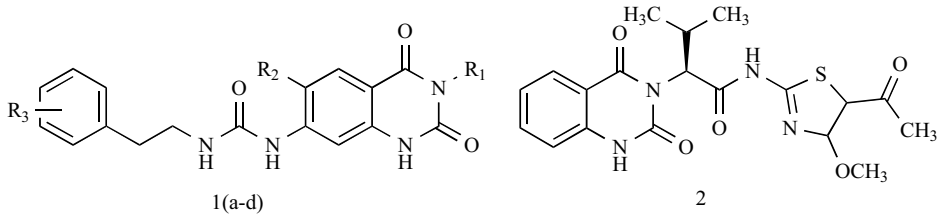


Fig. (1). Structures of some commercially available drugs bearing quinazoline and quinazolinone skeleton.



a: R_1=2-chlorophenylethyl, R_2=methoxy, R_3=2-chloro
b: R_1=3-chlorophenylethyl, R_2=4-methylpiperazine-1-yl, R_3=3-chloro
c: R_1=2-chlorophenylethyl, R_2=4-methoxypiperazine-1-yl, R_3=3-chloro
d: R_1=phenyl, R_2=methoxy, R_3=2-chloro

Fig. (2). Some cytotoxic 2,4(1*H*,3*H*)-quinazolinedione derivatives.

leading to the formation of corresponding 2-(3-ethoxycarbonylmethylureido)benzoic acid derivatives. Reactions were carried out by stirring the reactants in saturated potassium bicarbonate solution at room temperature to give moderate yields of urea derivatives (30-54%, **1**-**3**). Spectral data and melting point of the compound **1** were in accordance with the values of published before [60].

2-(3-Ethoxycarbonylmethylureido)benzoic acid derivatives (**1**-**3**) were refluxed in concentrated hydrochloric acid for 2 hours to give 2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid derivatives (**4**-**6**) in moderate to good yields of 36-73%. The compounds were easily seperated from the reaction media without using any purification techniques. Ring closure reaction was carried out by heating compound **1**-**3** in acidic media, as well as the ester functional group were hydrolysed to carboxylic acid to yield compound **4**-**6**.

Final compounds (**7**-**34**) were prepared by the amidation reaction of 2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic

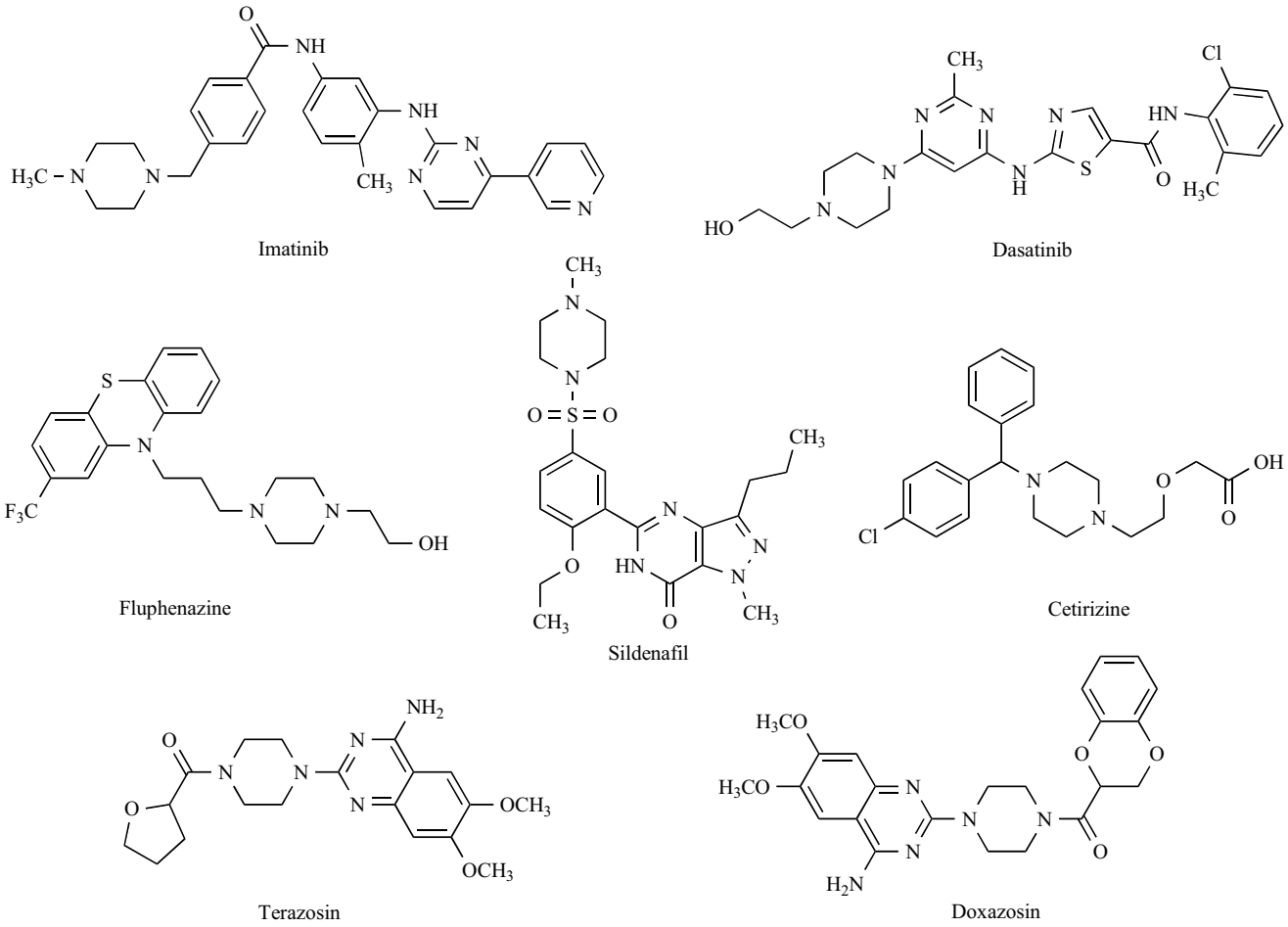


Fig. (3). Some clinically used piperazine derivatives.

acid derivatives (**4**-**6**) with various 1-substitutedpiperazines. Reactants were stirred with the coupling reagent *N,N′*-dicyclohexylcarbodiimide (DCC) in nitrogen atmosphere at cold (0-5 °C) to room temperature for 10-16 hours. Reaction solvent was evaporated to dryness. The residue was dissolved in hot acetonitrile then cooled in refrigerator to get the *N,N′*-dicyclohexylurea (DCU) precipitated. White crystalline DCU was removed by filtration. The liquid part was evaporated and crystallized from appropriate solvents. Compounds **7**-**34** were obtained in varied yields (5-84%).

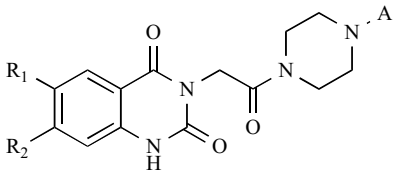


Fig. (4). General formula of target compounds.

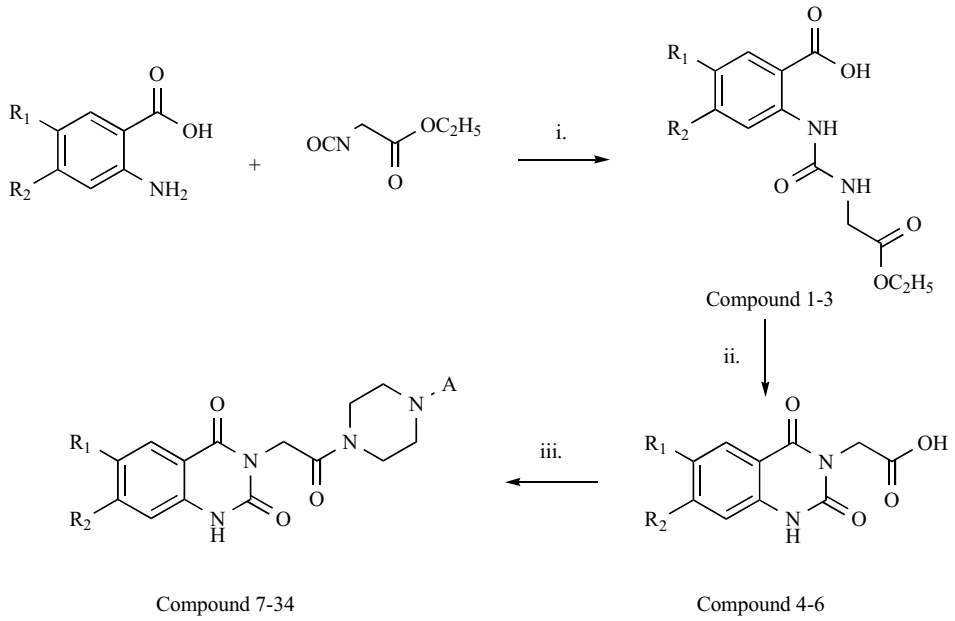
2.2. Tumor Cell Growth Inhibition Studies

All synthesized compounds were examined for their cytotoxic activity against hepatoma (HUH-7), breast cancer (MCF-7) and colorectal cancer (HCT-116) cell line with using Sulforhodamine B assay. According to the cytotoxicity data, some compounds exhibited cytotoxic activitiy between the values of 2.5-19.0 μM (Table **1**).

3-{2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1*H*,3*H*)-dione (compound **7**) presented the highest activity against HUH-7, MCF-7 and HCT-116 with the IC_{50} values of 2.5, 6.8 and 4.9 μM, respectively. 6-Chloro derivative (compound **15**) of this compound exhibited lower activity against these three cell lines than compound **7** with IC_{50} values of 7.0 μM, 13.1 μM and 9.4 μM, whereas 6,7-dimethoxy derivative (compound **25**) showed moderate cytotoxicity against HCT116 with IC_{50} value of 16.9 μM and no cytotoxicity against HUH-7 and MCF-7 cell lines (Table **2**).

Compound **8** presented cytotoxicity over HUH-7, MCF-7 and HCT-116 cell lines with IC_{50} values of 11.5, 12.2 and 35.3 μM, respectively. But 6-chloro (compound **17**) and 6,7-dimethoxy derivative (compound **27**) of this compound did not show any activity against these three cell lines.

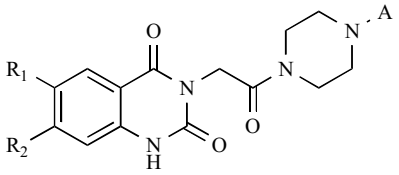
Compound **16** bearing 6-chloro atom on the 2,4-quinazolinedione ring exhibited moderate activity against HUH-7 and MCF-7 cell lines with IC_{50} values of 12.8 and



i. saturated $KHCO_3$ solution, rt, 1h.
ii. concd. HCl, reflux, 2h.
iii. 1-substitutedpiperazine, DCC, DCM, 0 °C (0.5h)- rt (10-16h)

Scheme (1). General synthetic pathway of the target compounds **7**-**34**.

Table 1. IC_{50} values for tested compounds 7-34 against hepatoma cell line (HUH-7), breast cancer cell line (MCF-7) and colorectal cancer cell line (HCT-116) using Sulforhodamine B assay. ([a]Camptothecin was positive control, [b]5-fluorouracil was reference drug, -: no inhibition).

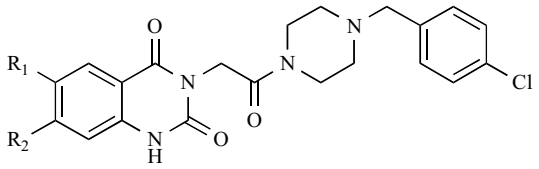


	R_1	R_2	A	IC_{50} Values (μM)		
				HUH-7	MCF-7	HCT-116
7	-H	-H	4-chlorobenzyl	2.5	6.8	4.9
8	-H	-H	1,3-benzodioxol-5-ylmethyl	11.5	12.2	35.3
9	-H	-H	2-furoyl	-	-	-
10	-H	-H	cyclohexyl	-	-	-
11	-H	-H	2-cyanophenyl	-	-	-
12	-H	-H	diphenylmethyl	-	15.2	-
13	-H	-H	benzoyl	-	-	-
14	-H	-H	4-pyridyl	-	-	-
15	-Cl	-H	4-chlorobenzyl	7.0	13.1	9.4
16	-Cl	-H	3-trifluoromethylphenyl	12.8	18.6	-

Table **1**. contd…

	R_1	R_2	A	IC_{50} Values (μM)		
				HUH-7	MCF-7	HCT-116
17	-Cl	-H	1,3-benzodioxol-5-ylmethyl	-	-	-
18	-Cl	-H	2-furoyl	-	-	-
19	-Cl	-H	cyclohexyl	-	-	-
20	-Cl	-H	2-cyanophenyl	-	-	-
21	-Cl	-H	diphenylmethyl	9.2	13.0	9.0
22	-Cl	-H	4-methoxyphenyl	-	-	-
23	-Cl	-H	benzoyl	-	-	-
24	-Cl	-H	4-pyridyl	-	-	-
25	$-OCH_3$	$-OCH_3$	4-chlorobenzyl	-	-	16.9
26	$-OCH_3$	$-OCH_3$	3-trifluoromethylphenyl	-	-	6.0
27	$-OCH_3$	$-OCH_3$	1,3-benzodioxol-5-ylmethyl	-	-	-
28	$-OCH_3$	$-OCH_3$	2-furoyl	-	-	-
29	$-OCH_3$	$-OCH_3$	cyclohexyl	-	-	11.4
30	$-OCH_3$	$-OCH_3$	2-cyanophenyl	-	-	19.0
31	$-OCH_3$	$-OCH_3$	diphenylmethyl	-	-	9.9
32	$-OCH_3$	$-OCH_3$	4-methoxyphenyl	-	-	-
33	$-OCH_3$	$-OCH_3$	benzoyl	-	-	-
34	$-OCH_3$	$-OCH_3$	4-pyridyl	-	-	-
Camptothecin[a]				0.00131	2.4×10^{-6}	9.2×10^{-7}
5-Flourouracil[b]				30.70	3.5	18.78

Table 2. IC_{50} values (μM) of compounds 7, 15 and 25.

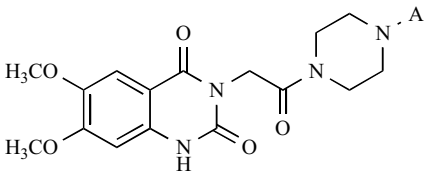


Compound	R_1	R_2	HUH-7	MCF-7	HCT-116
7	-H	-H	2.5	6.8	4.9
15	-Cl	-H	7.0	13.1	9.4
25	$-OCH_3$	$-OCH_3$	-	-	16.9

18.6 μM, whereas no activity against these cell lines was observed for the 6,7-dimethoxy derivative (compound **26**). Compound **26** only presented cytotoxic activity against HCT-116 with IC_{50} value of 6.0 μM.

Diphenylmethyl derivative of 6-chloro-2,4-quinazolinedione (compound **21**) showed cytotoxicity against HUH-7, MCF-7 and HCT-116 cell lines with IC_{50} values of 9.2 μM, 13 μM and 9 μM, respectively. Its non-substituted derivative

Table 3. IC_{50} values (μM) of compound 25, 26 and 29-31.



Compound	A	HUH-7	MCF-7	HCT-116
25	4-chlorobenzyl	-	-	16.9
26	3-(trifluoromethyl)phenyl	-	-	6.0
29	cyclohexyl	-	-	11.4
30	2-cyanophenyl	-	-	19.0
31	diphenylmethyl	-	-	9.9

(compound **12**) only exhibited cytotoxicity over MCF-7 cell line with IC_{50} value of 15.2 μM, whereas 6,7-dimethoxy derivative (compound **31**) only showed cytotoxicity over HCT-116 cell line with IC_{50} value of 9.9 μM.

Only the compounds **25**, **26** and **29-31** presented IC_{50} values ranging from 6 to19 μM against HCT-116 cell line. But generally, 6,7-dimethoxy derivatives (compound **25-34**) was non-cytotoxic over HUH-7, MCF-7 cell lines (Table **3**).

3. MATERIALS AND METHODS

3.1. Chemical Methods

Infrared Spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1), using potassium bromide pellets, the frequencies were expressed in cm^{-1}. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA), using tetramethylsilane (TMS) as the internal reference, with deuterated-dimethyl sulfoxide (DMSO-d_6) as solvent, the chemical shifts were reported in parts per million (ppm). Elemental analyses were performed on LECO 932 CHNS (Leco-932, St. Joseph, MI, USA) instrument. Liquid chromatography-mass spectrometry (LC-MS) spectra were recorded with a Waters 2695 Alliance Micromass ZQ instrument using electrospray ionization (ESI) technique.

General procedure for compounds 1-3. A solution of 2-aminobenzoic acid derivative (0.026 mol) in 35 ml of saturated potassium bicarbonate ($KHCO_3$) solution was stirred with 3.3 ml of ethyl isocyanatoacetate for an hour at room temperature. The solution was acidified with concentrated hydrochloric acid and filtered. The precipitate was crystallized from ethanol.

2-[3-(2-Ethoxy-2-oxoethyl)ureido]benzoic acid (1). 2-Aminobenzoic acid (7.056 g, 0.026 mol) and ethyl isocyanatoacetate (3.3 ml) were reacted according to the general procedure for compounds **1-3**. White needle crystals [61,62]; mp: 170 °C (171-172.5 °C [60]), yield: 2.117 g (30%). IR: (KBr, ν_{max}, cm^{-1}): 3398, 2993, 1725, 1683, 1663, 1536. ^{1}H-NMR: (400 MHz) (DMSO-d_6/TMS, ppm): δ 1.17-1.19 (t, 3H, CH_3, J=6.8 Hz), 3.8 (dd, 2H, NHCH_2), 4.1 (q, 2H, CH_2CH_3), 6.95 (m, 1H, benzene CH), 7.45 (m, 1H, benzene CH), 7.85 (t, 1H, CO*NH*CH_2), 7.9 (dd, 1H, benzene CH), 8.35 (d, 1H, benzene CH), 10.2 (s, 1H, CNHCO), 13.2 (s, 1H, COOH).

5-Chloro-2-[3-(2-ethoxy-2-oxoethyl)ureido]benzoic acid (2). 2-Amino-5-chlorobenzoic acid (4.547 g, 0.026 mol) and ethyl isocyanoacetate (3.3 ml) were reacted according to the general procedure for compounds **1-3**. Light yellow needle crystals; mp: 178.9 °C, yield: 2.950 g (37%). IR: (KBr, ν_{max}, cm^{-1}): 3336, 3041, 2980, 2928, 1732, 1690, 1650. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, ppm): δ 1.18 (t, 3H, CH_3, J=7.2 Hz), 3.81 (d, 2H, NHCH_2CO, J=6.0 Hz), 4.10 (q, 2H, CH_2CH_3, J=7.2 Hz), 7.53 (q, 1H, benzene CH, J=8.8 Hz, J=2.8 Hz), 7.83 (d, 1H, benzene CH, J=2.4 Hz), 7.95 (s, 1H, CO*NH*CH_2), 8.37 (d, 1H, benzene CH, J=8.8 Hz), 10.16 (s, 1H, CNHCO), 13.60 (s, 1H, COOH). Anal. calc. for $C_{12}H_{13}ClN_2O_5$ (300.6948); C, 47.93; H, 4.36; N, 9.32. Found: C, 47.53; H, 4.00; N, 9.39.

2-[3-(2-Ethoxy-2-oxoethyl)ureido]-4,5-dimethoxybenzoic acid (3). 2-Amino-4,5-dimethoxybenzoic acid (5.226 g, 0.026 mol) and ethyl isocyanoacetate (3.3 ml) were reacted according to the general procedure for compounds **1-3**. Light brown colored needle shaped crystals; mp: 172.5 °C, yield: 4.688 g, (54%). IR (KBr, ν_{max}, cm^{-1}): 3397, 3020, 2912, 1738, 1684, 1615, 1539. ^{1}H-NMR (400 MHz) (DMSO-d_6): 1.20 (t, 3H, OCH$_2$*CH$_3$*, J=7.2 Hz), 3.73 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.82 (d, 2H, NH*CH$_2$*, J=6.0 Hz), 4.11 (q, 2H, OCH_2CH_3, J=7.2 Hz), 7.37 (s, 1H, benzene CH), 7.92 (s, 1H, *NH*CH_2), 8.16 (s, 1H, benzene CH), 10.34 (s, 1H, C*NH*CO), 13.07 (s, 1H, COOH). Anal. calc. for $C_{14}H_{18}N_2O_7.1/2H_2O$: C, 50.15; H, 5.71; N, 8.35. Found: C, 49.73; H, 5.30; N, 8.39.

General procedure for compounds 4-6. Compounds **1-3** (0.011 mol) and 30 ml of concentrated hydrochloric acid were refluxed for 2 hours. The mixture was cooled and diluted with water to give compounds **4-6**.

2-(2,4-Dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetic acid (4). Compound **1** (3 g, 0.011 mol) was reacted according to the general procedure for compounds **1**-**3**. White powdered compound; mp: 295 °C (290-292 °C [63], 297-299 °C [64]), yield: 1.742 g (73%). IR (KBr, ν_{max}, cm^{-1}): 3285, 3010, 2945, 1716, 1657, 1625, 1494. ^{1}H-NMR (400 MHz) (DMSO-d_6): 4,56 (s, 2H, CH_2), 7.21-7.27 (m, 2H, benzene CH), 7.87-7.73 (m, 1H, benzene CH), 7.95 (dd, 1H, benzene CH, *J*=8.0 Hz, *J*=1.2 Hz), 11.62 (s, 1H, NH), 13.35 (s, 1H, COOH).

2-(6-Chloro-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetic acid (5). Compound **2** (3.4 g, 0.011 mol) was reacted according to the general procedure for compounds **1**-**3**. Light yellow needle shaped crytalline compound; mp: 300 °C (dec) (327-329 °C [64,65]), yield: 1.027 g (36%). IR (KBr, ν_{max}, cm^{-1}): 3071, 2930, 1716, 1656. ^{1}H-NMR (400 MHz) (DMSO-d_6): 4.56 (s, 2H, CH_2), 7.25 (d, 1H, benzene CH, *J*=8.8 Hz), 7.77 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.8 Hz), 7.89 (d, 1H, benzene CH, *J*=2.8 Hz), 11.78 (s, 1H, NH), 13.09 (s, 1H, COOH). Anal. calc. for $C_{10}H_7ClN_2O_4$: C, 47.17; H, 2.77; N, 11.00. Found: C, 47.13; H, 3.06; N, 10.98.

2-(6,7-Dimethoxy-2,4-dioxo-1,2-dihydroquinazolin-3(4-H)-yl)acetic acid (6). Compound **3** (3.69 g, 0.0113 mol) was reacted according to the general procedure for compounds **1**-**3**. Light creamy colored powdered crystalline compound; mp: 300 °C (dec), yield: 1.251 g (40%). IR (KBr, ν_{max}, cm^{-1}): 3017, 2959, 1702, 1620, 1513, 1468. ^{1}H-NMR (400 MHz) (DMSO-d_6): 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.54 (s, 2H, CH_2), 6.71 (s, 1H, benzene CH), 7.29 (s, 1H, benzene CH), 11.38 (s, 1H, NH), 12.96 (s, 1H, COOH). Anal. calc. for $C_{12}H_{12}N_2O_6$: C, 51.43; H, 4.32; N, 10.00. Found: C, 51.24; H, 4.03; N, 9.94.

General procedure for compounds 7-34. A mixture of compound **4-6** (1 mmol) in 5 ml of dry DCM and piperazine derivative (1 mmol) was cooled in an ice bath. Then, 1.1 mmol of DCC in 5 ml of dry dichloromethane was added to the mixture under nitrogen (N_2) atmosphere. Reaction mixture was stirred for 0.5 hour in an ice bath, then 10-16 hours at room temperature. Reaction solvent was evaporated to the dryness. Residue was dissolved in hot acetonitrile then cooled in refrigerator to get the DCU precipitated. White crystalline DCU was removed by filtration. Liquid part was evaporated and crystallized from appropriate solvents to give compound **7**-**34**

3-{2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1H,3H)-dione (7). Compound **4** (0.220 g, 1 mmol) and 1-(4-chlorobenzyl)piperazine (0.211 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from methanol-ether mixture to give compound **7** as white powder; mp: 300 °C (dec), yield: 91 mg (11%).). IR (KBr, ν_{max}, cm^{-1}): 3276, 3030, 2928, 2850, 1729, 1639, 1491. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.34 (t, 2H, piperazine CH_2, *J*=4.4 Hz), 2.43 (t, 2H, piperazine CH_2, *J*=4.4 Hz), 3.44 (t, 2H, piperazine CH_2, *J*=4.4 Hz), 3.52 (s, 2H, benzyl CH_2), 3.57 (t, 2H, piperazine CH_2, *J*=4.4 Hz), 4.74 (s, 2H, CH_2CO), 7.20-7.24 (m, 2H, benzene CH), 7.35-7.42 (dd, 4H, *J*=8.4 Hz, *J*=1.6 Hz, benzyl CH), 7.67-7.71 (m, 1H, benzene CH), 7.90-7.93 (m, 1H, benzene CH), 11.53 (bs, 1H, NH). Anal. calc. for $C_{21}H_{21}ClN_4O_3$: C, 61.09; H, 5.13; N, 13.57. Found: C, 60.98; H, 5.013; N, 13.52.

3-{2-[4-(Benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1H,3H)-dione (8). Compound **4** (0.220 g, 1 mmol) and 1-piperonylpiperazine (0.220 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from ethanol to give compound **8** as white powdered compound; mp: 218.2 °C, yield: 28 mg (7%). IR (KBr, ν_{max}, cm^{-1}): 3292, 3071, 3009, 2928, 2850, 1731, 1643, 1494. ^{1}H-NMR (400 MHz) (DMSO-d_6): 2.32 (t, 2H, CH2 piperazine, *J*=4.8 Hz), 2.42 (t, 2H, CH_2 piperazine, *J*=4.8 Hz), 3.44 (m, 4H, piperazine CH_2 and OCH_2O), 3.56 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 4.73 (s, 2H, CH_2CO), 6.0 (s, 2H, $NCH_2C_6H_5$), 6.76-7.25 (m, 3H, CH benzodioxol), 7.20-7.25 (m, 2H, benzene CH), 7.67-7.71 (m, 1H, benzene CH), 7.90-7.93 (m, 1H, benzene CH), 11.53 (bs, 1H, NH). Anal. calc. for $C_{22}H_{22}N_4O_5$. 1/2 H_2O: C, 61.24; H, 5.37; N, 12.99. Found: C, 61.13; H, 5.064; N, 12.88.

3-{2-[4-(2-Furoyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1H,3H)-dione (9). Compound **4** (0.220 g, 1 mmol) and 1-(2-furoyl)piperazine (0.180 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from *n*-hexane-ethanol mixture to give light yellow powdered compound; mp: 228 °C, yield: 320 mg (84%). IR ν_{max} (cm^{-1}): 3327, 3061, 2928, 2851, 1718, 1655, 1625, 1492, 1244. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.54 (t, 4H, piperazine CH_2), 3.70 (t, 4H, piperazine CH_2), 4.80 (s, 2H, CH_2CO), 6.65 (t, 1H, furoyl CH, *J*=1.6 Hz), 7.05-7.06 (m, 1H, furoyl CH), 7.21-7.25 (m, 2H, benzene CH), 7.68-7.71 (m, 1H, benzene CH), 7.88-7.94 (m, 2H, benzene CH and furoyl CH), 11.55 (bs, 1H, NH). Anal. calc. for $C_{19}H_{18}N_4O_5$. 1/9 H_2O: C, 59.37; H, 4.78; N, 14.58. Found: C, 59.18; H, 4.20; N, 14.35.

3-[2-(4-Cyclohexylpiperazin-1-yl)-2-oxoethyl]quinazoline-2,4(1H,3H)-dione (10). Compound **4** (0.220 g, 1 mmol) and 1-cyclohexylpiperazine (0.168 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from methanol-ether mixture to give white powdered compound; mp: 260.1 °C, yield: 23 mg (6%). IR (KBr, ν_{max}, cm^{-1}): 3327, 3060, 2928, 2852, 1719, 1657, 1623, 1493. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 1.06-1.21 (m, 5H, cyclohexyl CH_2), 1.54-1.72 (m, 5H, cyclohexyl CH_2), 2.26-2.31 (m, 1H, cyclohexyl CH), 2.42 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 2.52 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.38 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.49 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 4.70 (s, 2H, CH_2CO), 7.17-7.22 (m, 2H, benzene CH), 7.64-7.68 (m, 1H, benzene CH), 7.89 (dd, 1H, benzene CH, *J*=7.6 Hz, *J*=1.6 Hz), 11.49 (bs, 1H, NH). Anal. calc. for $C_{20}H_{26}N_4O_3$: C, 64.84; H, 7.07; N, 15.12. Found: C, 65.26; H, 7.28; N, 15.35.

2-{4-[(2,4-Dioxo-1,4-dihydroquinazolin-3(2H)-yl)acetyl]piperazin-1-yl}benzonitrile (11). Compound **4** (0.220 g, 1 mmol) and 1-(2-cyanophenyl)piperazine (0.187 g, 1 mmol)

were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from methanol-ether mixture to give yellow powdered compound; mp: 256.4 °C, yield: 173 mg (45%). IR (KBr, ν_{max}, cm^{-1}): 3273, 3072, 3003, 2962, 2872, 2214, 1727, 1670, 1640, 1489, 1455. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.13 (t, 2H, piperazine CH_2), 3.25 (t, 2H, piperazine CH_2), 3.63 (t, 2H, piperazine CH_2), 3.77 (t, 2H, piperazine CH_2), 4.81 (s, 2H, CH_2CO), 7.13 (t, 1H, 2-cyanophenyl CH, *J*=7.6 Hz), 7.19-7.23 (m, 3H, 2-cyanophenyl CH and benzene CHs), 7.59-7.73 (m, 3H, 2-cyanophenyl CH and benzene CH), 7.92 (dd, 1H, benzene CH, *J*=7.2 Hz, *J*=1.2 Hz), 11.47 (bs, 1H, NH). ^{13}C-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 41.91, 42.12, 44.77, 51.31, 51.98, 105.59, 113.90, 115.68, 118.57, 119.88, 122.95, 123.11, 127.85, 134.67, 134.84, 135.67, 139.88, 150.48, 155.32, 162.19, 165.41. MS (ESI^+, m/z): 390.2 ([M^+], base peak), 162.7 ($C_8H_6N_2O_2$, %10), 229.2 ($C_{13}H_{15}N_3O$, %12). Anal. calc. for $C_{21}H_{19}N_5O_3$: C, 64.77, H, 4.92; N, 17.98. Found: C, 65.54; H, 4.70; N, 17.64.

3-{2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1H,3H)-dione (12). Compound **4** (0.220 g, 1 mmol) and 1-(diphenylmethyl)piperazine (0.252 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from acetone-ether mixture to give white powdered compound; mp: 300 °C (dec), yield: 80 mg (18%). IR (KBr, ν_{max}, cm^{-1}): 3207, 3061, 3025, 2924, 2859, 2805, 1727, 1655, 1492. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.29 (t, 2H, piperazine CH_2), 2.37 (t, 2H, piperazine CH_2), 3.47 (t, 2H, piperazine CH_2), 3.60 (t, 2H, piperazine CH_2), 4.38 (s, 1H, CH), 4.71 (s, 2H, CH_2CO), 7.19-7.24 (m, 2H, benzene CH), 7.30-7.34 (m, 6H, diphenyl CH), 7.46 (d, 4H, diphenyl CH, *J*=7.6 Hz), 7.66-7.70 (m, 1H, benzene CH), 7.91 (q, 1H, benzene CH, *J*=8.0 Hz, *J*=1.2 Hz), 11.51 (bs, 1H, NH). Anal. calc. for $C_{27}H_{26}N_4O_3$. 1/3 H_2O: C, 70.42; H, 5.84; N, 12.17. Found: C, 70.37; H, 5.81; N, 12.12.

3-[2-(4-Benzoylpiperazin-1-yl)-2-oxoethyl]quinazoline-2,4(1H,3H)-dione (13). Compound **4** (0.220 g, 1 mmol) and 1-benzoylpiperazine (0.190 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from methanol-ether mixture to give white powdered compound; mp: 257 °C, yield: 110 mg (28%). IR (KBr, ν_{max}, cm^{-1}): 3274, 3065, 3001, 2959, 2911, 2860, 1726, 1676, 1617, 1492. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ ppm: 3.30-3.60 (m, 8H, piperazine CH_2), 4.79 (s, 2H, CH_2CO), 7.20-7.25 (m, 2H, benzene CH), 7.45-7.49 (m, 5H, benzoyl CH), 7.67-7.72 (m, 1H, benzene CH), 7.93 (dd, 1H, benzene CH, *J*=4 Hz, *J*=1.2 Hz), 11.55 (bs, 1H, NH). Anal. calc. for $C_{21}H_{20}N_4O_4$: C, 64.28; H, 5.14; N, 14.28. Found: C, 4.12; H, 4.75; N; 14.08.

3-[2-Oxo-2-(4-pyridin-4-ylpiperazin-1-yl)ethyl]quinazoline-2,4(1H,3H)-dione (14). Compound **4** (0.220 g, 1 mmol) and 1-(4-pyridyl)piperazine (0.163 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from methanol-ether mixture to give white powdered compound, mp: 300 °C (dec), yield: 24 mg (7%). IR (KBr, ν_{max}, cm^{-1}): 3178, 3089, 3003, 2944, 1708, 1659, 1514. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.35 (t, 2H, piperazine CH_2), 3.45 (t, 2H, piperazine CH_2), 3.58 (t, 2H, piperazine CH_2), 3.75 (t, 2H, piperazine CH_2), 4.80 (s, 2H, CH_2CO), 6.85 (dd, 2H, pyridine CH, *J*=4.0 Hz, *J*=1.2 Hz), 7.23 (m, 2H, benzene CH), 7.70 (m, 1H, benzene CH), 7.95 (dd, 1H, benzene CH), 8.20 (dd, 2H, pyridine CH), 11.55 (bs, 1H, NH). Anal. calc. for $C_{19}H_{19}N_5O_3$. 2/3 H_2O (365.3861 g/mol); C, 60.47; H, 5.43; N, 18.56. Found: C, 60.27; H, 5.49; N, 18.65.

3-{2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}-6-chloro-quinazoline-2,4(1H,3H)-dione (15). Compound 5 (0.255 g, 1 mmol) and 1-(4-chlorobenzyl)piperazine (0.211 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from methanol-ether mixture to give white powdered compound; mp: 300 °C (dec), yield: 39 mg (9%). IR (KBr, ν_{max}, cm^{-1}): 3503, 3060, 2916, 1726, 1662, 1457. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.32 (t, 2H, piperazine CH_2, *J*=4.4 Hz), 2.41 (t, 2H, piperazine CH_2, *J*=4.4 Hz), 3.42 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.50 (s, 2H, benzyl CH_2), 3.54 (t, 2H, piperazine CH_2, *J*=4.4 Hz), 4.71 (s, 2H, CH_2CO), 7.22 (d, 1H, benzene CH, *J*=9.2 Hz), 7.33-7.40 (m, 4H, benzyl CH), 7.75 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.8 Hz), 7.84 (d, 1H, benzene CH, *J*=2.4 Hz), 11.67 (bs, 1H, NH). Anal. calc. for $C_{21}H_{20}Cl_2N_4O_3$. 1/2 H_2O (447.3141 g/mol): C, 55.27; H, 4.64; N, 12.28. Found: C, 55.00; H, 4.55; N, 12.29.

6-Chloro-3-(2-oxo-2-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}ethyl) quinazoline-2,4(1H,3H)-dione (16). Compound **5** (0.255 g, 1 mmol) and 1-(3-trifluoromethylphenyl) piperazine (0,230 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from methanol-ether mixture to give white powdered compound; mp: 300 °C (dec), yield: 38 mg (9%). IR (KBr, ν_{max}, cm^{-1}): 3067, 2927, 1725, 1668, 1232, 1118, 1075. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.23 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.35 (t, 2H, piperazine CH_2), 3.59 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.73 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 4.80 (s, 2H, CH_2CO), 7.10 (d, 1H, 3-(trifluoromethyl)phenyl CH, *J*=7.2 Hz), 7.21-7.26 (m, 2H, 3-(trifluoromethyl)phenyl CH), 7.23 (d, 1H, benzene CH, *J*=8.8 Hz), 7.44 (t, 1H, 3-(trifluoromethyl)phenyl CH, *J*=8.0 Hz), 7.74 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.4 Hz), 7.85 (d, 1H, benzene CH, *J*=2.4 Hz), 11.70 (bs, 1H, NH). Anal. calc. for $C_{21}H_{18}ClF_3N_4O_3$ (466.8407 g/mol): C, 54.03; H, 3.89; N, 12.00. Found: C, 53.75; H, 3.77; N, 12.07.

3-{2-[4-(Benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl]-2-oxoethyl}-6-chloroquinazoline-2,4(1H,3H)-dione (17). Compound **5** (0.255 g, 1 mmol) and 1-piperonylpiperazine (0.220 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was washed with acetone and hot methanol to give white powdered compound; mp: 300 °C (dec), yield: 22 mg (5%). IR (KBr, ν_{max}, cm^{-1}): 3058, 2915, 1719, 1659, 1491. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.31 (t, 2H, piperazine CH_2), 2.40 (t, 2H, piperazine CH_2), 3.42 (s, 4H, piperazine CH_2 and OCH_2O), 3.53 (t, 2H, piperazine CH_2), 4.71 (s, 2H, CH_2CO), 5.97 (s, 2H, $NCH_2C_6H_3$), 6.74-6.87 (m, 3H,

benzodioxol CH), 7.22 (d, 1H, benzene CH, *J*=8.4 Hz), 7.72 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.4 Hz), 7.84 (d, 1H, benzene CH, *J*=2.0 Hz), 11.70 (bs, 1H, NH). Anal. calc. for $C_{22}H_{21}ClN_4O_5$. 2/3 H_2O (456.8788 g/mol): C, 54.61; H, 5.00; N, 11.58. Found: C, 54.46; H, 5.19; N, 11.25.

6-Chloro-3-{2-[4-(2-furoyl)piperazin-1-yl]-2-oxoethyl}-quinazoline-2,4(1H,3H)-dione (18). Compound **5** (0.255 g, 1 mmol) and 1-(2-furoyl)piperazine (0.180 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was washed with hot methanol to give white powdered compound; mp: 294.5 °C, yield: 89 mg (21%). IR (KBr, ν_{max}, cm^{-1}): 3054, 2931, 1718, 1655, 1486. ^{1}H-NMR (400 MHz) (DMSO-d_6 / TMS) δ ppm: 3.28 (t, 2H, piperazine CH_2), 3.52 (t, 2H, piperazine CH_2), 3.67-3.75 (m, 4H, piperazine CH_2), 4.78 (s, 2H, CH_2CO), 6.64 (dd, 1H, furoyl CH, *J*=3.6 Hz, *J*=2.0 Hz), 7.04 (d, 1H, furoyl CH, *J*=2.8 Hz), 7.23 (d, 1H, benzene CH, *J*=8.4 Hz), 7.74 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.4 Hz), 7.85-7.86 (m, 2H, benzene CH and furoyl CH), 11.75 (bs, 1H, NH). Anal. calc. for $C_{19}H_{17}ClN_4O_5$ (416.8149 g/mol): C, 53.97; H, 4.21; N, 13.25. Found: C, 54.18; H, 4.12; N, 13.35.

6-Chloro-3-[2-(4-cyclohexylpiperazin-1-yl)-2-oxoethyl] quinazoline-2,4(1H,3H)-dione (19). Compound **5** (0.255 g, 1 mmol) and 1-cyclohexylpiperazine (0.168 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was washed with hot methanol to give white powdered compound; mp: 300 °C (dec), yield: 36 mg (9%). IR (KBr, ν_{max}, cm^{-1}): 3068, 2925, 1722, 1660. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 1.16-1.22 (m, 5H, cyclohexyl), 1.54-1.73 (m, 5H, cyclohexyl), 2.26 (1H, m, cyclohexyl CH), 2.43 (t, 2H, piperazine CH_2, *J*=4.4 Hz), 2.53 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.39 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.50 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 4.71 (s, 2H, CH_2CO), 7.22 (d, 1H, benzene CH, *J*=8.8 Hz), 7.72 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.4 Hz), 7.83 (d, 1H, benzene CH, *J*=2.0 Hz), 11.68 (bs, 1H, NH). Anal. calc. for $C_{20}H_{25}ClN_4O_3$ .1/3 H_2O (404.8904 g/mol): C, 58.46; H, 6.30; N, 13.64. Found: C, 58.02; H, 6.00; N, 13.53.

2-{4-[2-(6-Chloro-2,4-dioxo-1,2-dihydroquinazolin-3(4H)-yl)acetyl]piperazin-1-yl}benzonitrile (20). Compound **5** (0.255 g, 1 mmol) and 1-(2-cyanophenyl)piperazine (0.187 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was washed with hot methanol to give white powdered compound; mp: higher than 300 °C, yield: 172 mg (41%). IR (KBr, ν_{max}, cm^{-1}): 3188, 3069, 2961, 2928, 2860, 2224, 1714, 1663, 1489. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.14 (t, 2H, piperazine CH_2), 3.24 (t, 2H, piperazine CH_2), 3.64 (t, 2H, piperazine CH_2), 3.78 (t, 2H, piperazine CH_2), 4.83 (s, 2H, CH_2CO), 7.15 (t, 1H, 2-cyanophenyl CH, *J*=7.6 Hz), 7.21-7.26 (m, 2H, benzene CH and 2-cyanophenyl CH), 7.62-7.66 (m, 1H, benzene CH), 7.74-7.78 (m, 2H, 2-cyanophenyl CH), 7.87 (d, 1H, benzene CH, *J*=2.8 Hz), 11.72 (s, 1H, NH). ^{13}C-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 42.06, 42.17, 44.81, 51.28, 51.94, 105.58, 115.33, 117.98, 119.88, 118.54, 122.92, 126.74, 127.12, 134.66, 134.80, 135.58, 138.76, 150.17, 155.29, 161.21, 165.22. MS (ESI^+, m/z): 426.2 ($[M^{+2}]^+$) 424.1 ($[M^+]$, base peak). Anal. calc. for $C_{21}H_{18}ClN_5O_3$. 1/5 H_2O (423.8522 g/mol): C, 59.01; H, 4.34; N, 16.38. Found: C, 58.97; H, 4.26; N, 16.30.

6-Chloro-3-{2-[4-(diphenylmethyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1H,3H)-dione (21). Compound **5** (0.255 g, 1 mmol) and 1-(diphenylmethyl)piperazine (0.252 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was washed with hot methanol to give white powdered compound; mp: 300 °C (dec), yield: 45 mg (18%). IR (KBr, ν_{max}, cm^{-1}): 3187, 3062, 2851, 2928, 1727, 1655, 1491. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.29 (t, 2H, piperazine CH_2), 2.37 (t, 2H, piperazine CH_2), 3.46 (t, 2H, piperazine CH_2), 3.59 (t, 2H, piperazine CH_2), 4.38 (s, 1H, CH), 4.71 (s, 2H, CH_2CO), 7.19-7.24 (m, 1H, benzene CH), 7.30-7.34 (m, 6H, diphenyl CH), 7.45-7.47 (m, 4H, diphenyl CH), 7.74 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.4 Hz), 7.86 (d, 1H, benzene CH, *J*=2.8 Hz), 11.69 (bs, 1H, NH). Anal. calc. for $C_{27}H_{25}ClN_4O_3$. 2/3 H_2O (488.9653 g/mol): C, 64.73; H, 5.30; N, 11.18. Found: C, 64.86; H, 5.29; N, 10.85.

6-Chloro-3-{2-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1H,3H)-dione (22). Compound **5** (0.255 g, 1 mmol) and 1-(4-methoxyphenyl)piperazine (0.192 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was washed with acetone and hot methanol to give white powdered compound; mp: 300 °C (dec), yield: 102 mg (24%). IR (KBr, ν_{max}, cm^{-1}): 3067, 2928, 1724, 1655, 1512. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.97 (t, 2H, piperazine CH_2), 3.01 (t, 2H, piperazine CH_2), 3.57 (t, 2H, piperazine CH_2), 3.68 (s, 3H, OCH_3), 3.70 (t, 2H, piperazine CH_2), 4.78 (s, 2H, CH_2CO), 6.82-6.94 (m, 4H, methoxyphenyl CH), 7.23 (d, 1H, benzene CH, *J*=8.8 Hz), 7.73 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.4 Hz), 7.84 (d, 1H, benzene CH, *J*=2.8 Hz), 11.63 (bs, 1H, NH). Anal. calc. for $C_{21}H_{21}ClN_4O_4$. 2/3 H_2O (428.8687g/mol): C, 57.21; H, 5.11; N, 12.71. Found: C, 56.95; H, 4.84; N, 12.68.

3-[2-(4-Benzoylpiperazin-1-yl)-2-oxoethyl]-6-chloroquinazoline-2,4(1H,3H)-dione (23). Compound **5** (0.255 g, 1 mmol) and 1-benzoylpiperazine (0.190 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was washed with acetone and hot methanol to give white powdered compound; mp: 300 °C (dec), yield: 35 mg (8%). IR (KBr, ν_{max}, cm^{-1}): 3057, 2930, 1715, 1660, 1618. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.50 (t, 4H, piperazine CH_2), 3.63 (t, 4H, piperazine CH_2), 4.76 (s, 2H, CH_2CO), 7.23 (d, 1H, benzene CH, *J*=8.8 Hz), 7.42-7.46 (m, 5H, benzoyl CH), 7.73 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.4 Hz), 7.84 (d, 1H, benzene CH, *J*=2.4 Hz), 11.64 (bs, 1H, NH). Anal. calc. for $C_{21}H_{19}ClN_4O_4$. 2/3 H_2O (426.8528 g/mol): C, 57.47; H, 4.67; N, 12.77. Found: C, 57,05; H, 4,62; N, 12,69.

6-Chloro-3-[2-oxo-2-(4-pyridin-4-ylpiperazin-1-yl)ethyl]quinazoline-2,4(1H,3H)-dione (24). Compound **5** (0.255 g, 1 mmol) and 1-(4-pyridyl)piperazine (0.163 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was crystallized from methanol-ether mixture to give white powdered compound; mp: 300 °C (dec), yield: 24 mg (6%). IR (KBr, ν_{max}, cm^{-1}): 3068, 2932, 1715, 1657, 1595. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.34 (t, 2H, piperazine CH_2), 3.44 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.56 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.71 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 4.80

(s, 2H, CH_2CO), 6.83 (dd, 2H, pyridine CH, *J*=5.2 Hz, *J*=1.2 Hz), 7.23 (d, 1H, benzene CH, *J*=8.4 Hz), 7.75 (dd, 1H, benzene CH, *J*=8.8 Hz, *J*=2.4 Hz), 7.85 (d, 1H, benzene CH, *J*=2.8 Hz), 8.18 (d, 2H, pyridine CH, *J*=6.8 Hz), 11.70 (bs, 1H, NH). Anal. calc. for $C_{19}H_{18}ClN_5O_3$ (399.8308 g/mol): C, 57.07; H, 4.54; N, 17.52. Found: C, 57.49; H, 4.45; N, 17.13.

3-{2-[4-(4-Chlorobenzyl)piperazin-1-yl]-2-oxoethyl}-6,7-dimethoxyquinazoline-2,4(1H,3H)-dione (25). Compound **6** (0.280 g, 1 mmol) and 1-(4-chlorobenzyl)piperazine (0.211 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was crystallized from methanol-ether mixture to give white powdered crystalline compound; mp: 300 °C (dec), yield: 25 mg (5%). IR (KBr, v_{max}, cm^{-1}): 3096, 2955, 1718, 1657, 1513. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.30 (t, 2H, piperazine CH_2), 2.40 (t, 2H, piperazine CH_2), 3.41 (t, 2H, piperazine CH_2), 3.49 (s, 2H, benzyl CH_2), 3.54 (t, 2H, piperazine CH_2), 3.76 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.68 (s, 2H, CH_2CO), 6.68 (s, 1H, benzene CH), 7.24 (s, 1H, benzene CH), 7.35 (dd, 4H, benzyl CH, *J*=17.6 Hz, *J*=8.8 Hz), 11.27 (s, 1H, NH). Anal. calc. for $C_{23}H_{25}ClN_4O_5$. 2/3 H_2O (472.9213 g/mol): C, 56.97; H, 5.47; N, 11.55. Found: C, 56.88; H, 5.85; N, 11.38.

6,7-Dimethoxy-3-(2-oxo-2-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}ethyl)quinazoline-2,4(1H,3H)-dione (26). Compound **6** (0.280 g, 1 mmol) and 1-(3-trifluoromethylphenyl)piperazine (0,230 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was crystallized from methanol-ether mixture to give white powdered crystalline compound; mp: 228 °C, yield: 41 mg (8%). IR (KBr, v_{max}, cm^{-1}): 3079, 2958, 1705, 1671, 1646, 1512. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.24 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.32 (t, 2H, piperazine CH_2), 3.60 (t, 2H, piperazine CH_2), 3.75 (t, 2H, piperazine CH_2), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.80 (s, 2H, CH_2CO), 6.71 (s, 1H, benzene CH), 7.12 (d, 1H, 3-(trifluoromethyl)phenyl CH, *J*=8.0 Hz), 7.23 (s, 1H, benzene CH), 7.26-7.29 (m, 2H, 3-(trifluoromethyl)phenyl CH), 7.46 (t, 1H, 3-(trifluoromethyl)phenyl CH, *J*=8.0 Hz), 11.34 (s, 1H, NH). Anal. calc. for $C_{23}H_{23}F_3N_4O_5$ (492.4479 g/mol): C, 56.10; H, 4.71; N, 11.38. Found: C, 56.13; H, 4.80; N, 11.30.

3-{2-[4-(Benzo[d][1,3]dioxol-5-ylmethyl)piperazin-1-yl]-2-oxoethyl}-6,7-dimethoxy quinazoline-2,4(1H,3H)-dione (27). Compound **6** (0.280 g, 1 mmol) and 1-piperonylpiperazine (0.220 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was crystallized from methanol-ether mixture to give white powdered crystalline compound; mp: 211.3 °C, yield: 64 mg (15%). IR (KBr, v_{max}, cm^{-1}): 3092, 2946, 1709, 1662, 1469. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.31 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 2.41 (t, 2H, piperazine CH_2), 3.31 (t, 2H, piperazine CH_2), 3.43 (s, 2H, OCH_2O), 3.55 (t, 2H, piperazine CH_2), 3.79 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2CO), 6.00 (s, 2H, NCH_2-benzene), 6.70 (s, 1H, benzene CH), 6.76-6.89 (m, 3H, benzodioxol CH), 7.27 (s, 1H, benzene CH), 11.30 (s, 1H, NH). Anal. calc. for $C_{22}H_{22}N_4O_5$ (422.434 g/mol): C, 59.74; H, 5.43; N, 11.61. Found: C, 59.87; H, 5.20; N, 11.59.

3-{2-[4-(2-Furoyl)*piperazin-*1-yl]-2-oxoethyl}-6,7-dimethoxyquinazoline-2,4(1H,3H)-dione (28). Compound **6** (0.280 g, 1 mmol) and 1-(2-furoyl)piperazine (0.180 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was crystallized from ethanol to give white powdered crystalline compound; mp: 300 °C (dec), yield: 79 mg (18%). IR (KBr, v_{max}, cm^{-1}): 3017, 2934, 1714, 1658, 1624, 1514. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS) δ ppm: 3.54 (t, 4H, piperazine CH_2), 3.70 (t, 4H, piperazine CH_2), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.78 (s, 2H, CH_2CO), 6.65-6.66 (m, 1H, furoyl CH), 6.71 (s, 1H, benzene CH), 7.06 (d, 1H, furoyl CH, *J*=3.6 Hz), 7.28 (s, 1H, benzene CH), 7.88 (d, 1H, furoyl CH, *J*=0.8 Hz), 11.34 (s, 1H, NH). Anal. calc. for $C_{21}H_{22}N_4O_7.3H_2O$ (442.4221 g/mol): C, 50.80; H, 5.68; N, 11.29. Found: C, 51.08; H, 5.77; N, 11.34.

3-[2-(4-Cyclohexylpiperazin-1-yl)-2-oxoethyl]-6,7-dimethoxyquinazoline-2,4(1H,3H)-dione (29). Compound **6** (0.280 g, 1 mmol) and 1-cyclohexylpiperazine (0.168 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was crystallized from methanol-ether mixture to give white powdered crystalline compound; mp: 239.7 °C, yield: 74 mg (17%). IR (KBr, v_{max}, cm^{-1}): 3084, 3008, 2926, 2856, 1702, 1661, 1512. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 1.09-1.74 (m, 10H, cyclohexyl CH_2), 2.30 (m, 1H, cyclohexyl CH), 2.45 (t, 2H, piperazine CH_2, *J*=4.0 Hz), 2.54 (t, 2H, piperazine CH_2), 3.41 (t, 2H, piperazine CH_2, *J*=3.6 Hz), 3.52 (t, 2H, piperazine CH_2), 3.79 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2CO), 6.70 (s, 1H, benzene CH), 7.26 (s, 1H, benzene CH), 11.30 (s, 1H, NH). Anal. calc. for $C_{22}H_{30}N_4O_5$. 1/4 H_2O (430.4976 g/mol): C, 60.74; H, 7.07; N, 12.88. Found: C, 60.57; H, 7.19; N, 12.79.

2-{4-[(6,7-Dimethoxy-2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl)acetyl]piperazin-1-yl}benzonitrile (30). Compound **6** (0.280 g, 1 mmol) and 1-(2-cyanophenyl)piperazine (0.187 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was crystallized from methanol-ether mixture to give white powdered crystalline compound; mp: 300 °C (dec), yield: 21 mg (5%). IR (KBr, v_{max}, cm^{-1}): 3094, 2955, 2214, 1720, 1663, 1514. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ ppm): 3.13 (t, 2H, piperazine CH_2), 3.24 (t, 2H, piperazine CH_2), 3.64 (t, 2H, piperazine CH_2), 3.78 (t, 2H, piperazine CH_2), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.80 (s, 2H, CH_2CO), 6.72 (s, 1H, benzene CH), 7.15 (t, 1H, 2-cyanophenyl CH, *J*=7.6 Hz), 7.22 (d, 1H, 2-cyanophenyl CH, *J*=8.0 Hz), 7.28 (s, 1H, benzene CH), 7.62-7.66 (m, 1H, 2-cyanophenyl CH), 7.76 (dd, 1H, 2-cyanophenyl CH, *J*=8.0 Hz, *J*=1.6 Hz), 11.33 (s, 1H, NH). Anal. calc. for $C_{23}H_{23}N_5O_5$. H_2O (449.4594 g/mol): C, 59.09; H, 5.39; N, 14.98. Found: C, 58.85; H, 5.24; N, 14.84.

3-{2-[4-(Diphenylmethyl)piperazin-1-yl]-2-oxoethyl}-6,7-dimethoxyquinazoline-2,4(1H,3H)-dione (31). Compound **6** (0.280 g, 1 mmol) and 1-(diphenylmethyl) piperazine (0.252 g, 1 mmol) were reacted according to the general procedure for compounds **7-34** and the crude product was crystallized from methanol-ether mixture to give white powdered crystalline compound; mp: 262.4°C, yield: 21 mg

(4%). IR (KBr, ν_{max}, cm^{-1}): 3057, 2961, 2889, 1709, 1666, 1511. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.29 (t, 2H, piperazine CH_2), 2.36 (t, 2H, piperazine CH_2), 3.46 (t, 2H, piperazine CH_2), 3.60 (t, 2H, piperazine CH_2), 3.79 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.38 (s, 1H, diphenyl CH), 4.68 (s, 2H, CH_2CO), 6.70 (s, 1H, benzene CH), 7.20 (t, 2H, diphenylmethyl CH, *J*=7.2 Hz), 7.26 (s, 1H, benzene CH), 7.32 (t, 4H, diphenylmethyl CH, *J*=7.6 Hz), 7.46 (d, 4H, diphenylmethyl CH, *J*=7.2 Hz), 11.29 (s, 1H, NH). Anal. calc. for $C_{29}H_{30}N_4O_5$ (514.5725 g/mol): C, 67.69; H, 5.88; N, 10.89. Found: C, 67.33; H, 6.01; N, 11.01.

6,7-Dimethoxy-3-{2-[4-(4-methoxyphenyl)piperazin-1-yl]-2-oxoethyl}quinazoline-2,4(1H,3H)-dione (32). Compound **6** (0.280 g, 1 mmol) and 1-(4-methoxyphenyl) piperazine (0.192 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was washed with hot acetonitrile and methanol to give white powdered crystalline compound; mp: 283.5 °C, yield: 120 mg (26%). IR (KBr, ν_{max}, cm^{-1}): 3194, 3065, 3013, 2948, 2886, 2830, 1723, 1659, 1513. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 2.99 (t, 2H, piperazine CH_2, *J*=3.6 Hz), 3.09 (t, 2H, piperazine CH_2, *J*=3.6 Hz), 3.59 (t, 2H, piperazine CH_2, *J*=3.6 Hz), 3.70 (s, 3H, 4-methoxyphenyl OCH_3), 3.73 (t, 2H, piperazine CH_2, *J*=3.6 Hz), 3.80 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.79 (s, 2H, CH_2CO), 6.72 (s, 1H, benzene CH), 6.91 (dd, 4H, 4-methoxyphenyl CH), 7.28 (s, 1H, benzene CH), 11.33 (s, 1H, NH). ^{13}C-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 41.72, 42.05, 44.62, 50.27, 50.71, 55.62, 56.19, 56.31, 97.99, 105.98, 107.92, 114.75, 118.58, 135.63, 145.58, 145.68, 150.58, 153.85, 155.56, 161.69, 165.34. MS (ESI^+, m/z): 455.2 ([M^+], base peak), 207.2 ($C_{10}H_9NO_4$), 249.2 ($C_{13}H_{19}N_3O_2$), 262.9 ($C_{12}H_{11}N_2O_5$). Anal. calc. for $C_{23}H_{26}N_4O_6$. 1/4 H_2O (454.4759 g/mol): C, 60.19; H, 5.82; N, 12.21. Found: C, 60.17; H, 5.84; N, 12.20.

3-[2-(4-Benzoylpiperazin-1-yl)-2-oxoethyl]-6,7-dimethoxyquinazoline-2,4(1H,3H)-dione (33). Compound **6** (0.280 g, 1 mmol) and 1-benzoylpiperazine (0.190 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from methanol-ether mixture to give white powdered crystalline compound; mp: 168.5 °C, yield: 32 mg (7%). IR (KBr, ν_{max}, cm^{-1}): 3010, 2958, 1712, 1660, 1623, 1513. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.51 (t, 4H, piperazine CH_2), 3.66 (t, 4H, piperazine CH_2), 3.79 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.76 (s, 2H, CH_2CO), 6.71 (s, 1H, benzene CH), 7.27 (s, 1H, benzene CH), 7.45-7.49 (m, 5H, benzoyl CH), 11.32 (s, 1H, NH). Anal. calc. for $C_{23}H_{24}N_4O_6$. 3 H_2O (452.46 g/mol): C, 54.54; H, 5.97; N, 11.06. Found: C, 54.68; H, 5.99; N, 11.15.

6,7-Dimethoxy-3-[2-oxo-2-(4-pyridin-4-ylpiperazin-1-yl)ethyl]quinazoline-2,4(1H,3H)-dione (34). Compound **6** (0.280 g, 1 mmol) and 1-(4-pyridyl)piperazine (0.163 g, 1 mmol) were reacted according to the general procedure for compounds **7**-**34** and the crude product was crystallized from methanol-ether mixture to give white powdered crystalline compound; mp: 300 °C (dec), yield: 32 mg (8%). IR (KBr, ν_{max}, cm^{-1}): 3068, 2932, 1715, 1657, 1510. ^{1}H-NMR (400 MHz) (DMSO-d_6/TMS, δ, ppm): 3.33 (t, 2H, piperazine CH_2), 3.43 (t, 2H, piperazine CH_2, *J*=4.4 Hz), 3.54 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.70 (t, 2H, piperazine CH_2, *J*=4.8 Hz), 3.77 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.76 (s, 2H, CH_2CO), 6.68 (s, 1H, benzene CH), 6.82 (dd, 2H, pyridine CH, *J*=5.2 Hz, *J*=1.6 Hz), 7.25 (s, 1H, benzene CH), 8.16 (dd, 2H, pyridine CH, *J*=5.2 Hz, *J*=1.6 Hz), 11.30 (s, 1H, NH). Anal. calc. for $C_{21}H_{23}N_5O_5$. 2 H_2O (425.438 g/mol): C, 54.66; H, 5.90; N, 15.18. Found: C, 54.72; H, 5.79; N, 15.16.

3.2. Biological Methods

3.2.1. Anticancer Activity Test Procedure: Sulforhodamine B Assay

Cells were plated in 96-well plates (1000-5000 cell/well in 200 µl) and grown for 24 hours at 37 °C before being treated with various concentrations of the tested compounds (from 2.5 to 40 µM). After 72 hours of incubation the medium was aspirated, washed once with PBS ($CaCl_2$-, $MgCl_2$-free) (Gibco, Invitrogen), and then 50 µl of a cold (4 °C) solution of 10% (v/v) trichloroacetic acid (Merck) was added. Microplates were left for 1 hour at 4 °C. After aspiration of the solution, plates were washed five times with deionized water and left to dry. 50 µl of a 0.4% (w/v) of sulforhodamine B solution was removed and the plates were washed five times with 1% acetic acid before air-drying. Bound sulforhodamine B solubilize in a 200 µl 10 mM Tris-base solution and the plates were left on a plate shaker for 10 minutes. The absorbance was read in a 96-well plate reader at 515 nm.

CONCLUSION

In conclusion, synthesized compounds generally showed moderate or no *in vitro* cytotoxic activity against HUH-7, MCF-7 and HCT-116 cell lines. 4-Chlorobenzyl, 3-trifluoromethylphenyl and diphenyl derivatives generally exhibited better IC_{50} values when compered to others. For our further studies, 4-chlorobenzyl derivative (compound **7)** was selected as a promising lead in order to generate more effective anticancer agents. In addition, when the hydrogens at sixth and seventh position of the 2,4-(1*H*,3*H*)-quinazolinedione ring replaced by methoxy groups, cytotoxic activity did not increased but some selectivity was provided againts HCT-116 cell lines. This result will lead us to synthesize more selective derivatives in our future studies.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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