Taylor & Francis
Taylor & Francis Group

Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: informahealthcare.com/journals/ienz20

Quinazoline-sulfonamides as potential antitumor agents: synthesis and biological testing

Ahmed M. Alafeefy, Saleh I. Alqasoumi, Abdelkader E. Ashour & Mashael M. Alshebly

To cite this article: Ahmed M. Alafeefy, Saleh I. Alqasoumi, Abdelkader E. Ashour & Mashael M. Alshebly (2013) Quinazoline-sulfonamides as potential antitumor agents: synthesis and biological testing, Journal of Enzyme Inhibition and Medicinal Chemistry, 28:2, 375-383, DOI: 10.3109/14756366.2012.668541

To link to this article: https://doi.org/10.3109/14756366.2012.668541

Published online: 02 Apr 2012.

Journal of Enzyme Inhibition and Medicinal Chemistry, 2013; 28(2): 375–383

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.3109/14756366.2012.668541

informa
healthcare

RESEARCH ARTICLE

Quinazoline-sulfonamides as potential antitumor agents: synthesis and biological testing

Ahmed M. Alafeefy[1], Saleh I. Alqasoumi[2], Abdelkader E. Ashour[3], and Mashael M. Alshebly[4]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, Salman Bin Abdulaziz University, Al-Kharj, Saudi Arabia, [2]Department of Pharmacognosy, [3]Department of Pharmacology and Toxicology, and [4]Department of Obstetrics and Gynaecology, College of Medicine, King Saud University, Riyadh, Saudi Arabia

Abstract

New series of quinazoline containing sulfonamide derivatives were prepared and screened for their antitumor activity. Four human cancer cell lines, namely, hepatoma cancer cell line (HepG2), breast cancer cell line (MCF-7), cervix cancer cell line (HeLa) and colon cancer cell line (HCT-8), were used to measure the cytotoxic activity. Compounds **8** and **21** exhibited remarkable antitumor activity almost similar to that of the standard drug (doxorubicin). Six compounds **16**, **22**, **23**, **29**, **30** and **33**, showed considerable activity and few compounds were totally inactive.

Keywords: Antitumor, quinazoline, sulfonamide, synthesis

Introduction

Tumor growth and metastasis depends on multiple factors, including the physiological process of angiogenesis. For example, elevated expression of vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor and platelet derived growth factor are associated with tumor angiogenesis, metastases, survival and resistance to apoptosis. Therefore, these growth factors have represented potential molecular targets for inhibition of tumor growth and progression[1–4]. In addition, data accumulate that significant levels of cross talk exists within and between signalling networks in most tumours, necessitating the combined inhibition of multiple targets in order to establish efficient tumour growth inhibition. In that light, cell signalling network models indicate that partial inhibition of a number of targets is more effective than the complete inhibition of a single target[5]. Consequently, various approaches were pursued to inhibit multiple signalling pathways. The use of a combination of different compounds can introduce adverse effects related to pharmacokinetics, toxicity and patient compliance. Alternatively, the use of multi-targeted agents can circumvent most of the problems due to combination therapy[6].

Therefore, our interest was to develop a series of novel small molecules as potential antitumor agents that can block biologically relevant molecular targets. In this application, we prepared certain quinazoline containing sulfonamide pharmacophores and/or their isosteres. The quinazoline core has been shown to be a suitable carrier that allows for an effective DNA intercalation. In that context, several quinazoline derivatives have been shown to exhibit high affinity for receptor tyrosine kinases, as well as other oncogenic targets[5,6].

Many new sulfonamide derivatives, such as E7070 (indisulam), have shown substantial antitumor activity *via* different mechanisms[7–17]. It was also reported to selectively accumulate within cancerous cells. Therefore, in this work, we synthesized somequinazoline containing sulfonamide derivatives and studied their activity against some selected human tumour cell lines.

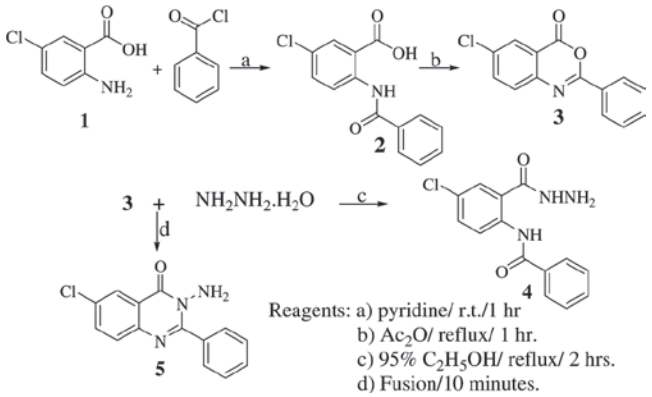
Results and discussion

Chemistry

The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in Schemes 1–3. In Scheme 1, 5-chloroanthranilic acid was

Address for Correspondence: Dr. Ahmed Mahmoud Alafeefy, Pharmaceutical Chemistry Department, College of Pharmacy, Salman Bin Abdulaziz University, B.O. Box 173, Al-Kharj 11942, Saudi Arabia. Tel: +966-507-069-896. E-mail: ahmed.alafeefy@yahoo.com
(Received 19 January 2012; revised 16 February 2012; accepted 17 February 2012)

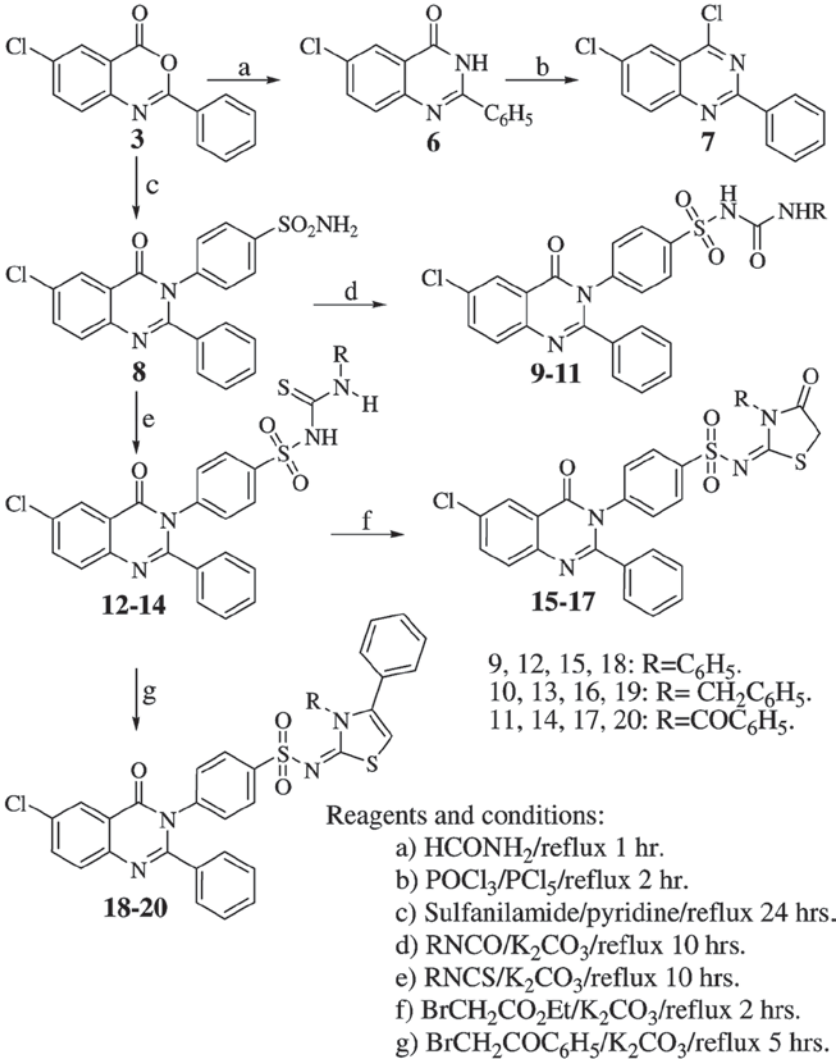
allowed to react with benzoyl chloride in pyridine to afford the amide derivative **2** that underwent dehydrative cyclization through boiling in acetic anhydride to afford the corresponding 3*H*-benzoxazin-4-one **3**. Treating the latter compound with hydrazine hydrate in 95% ethanol afforded the diamide derivative **4**. However, hydrazinolysis under the condition of fusion yielded the 3-amino derivative **5**.



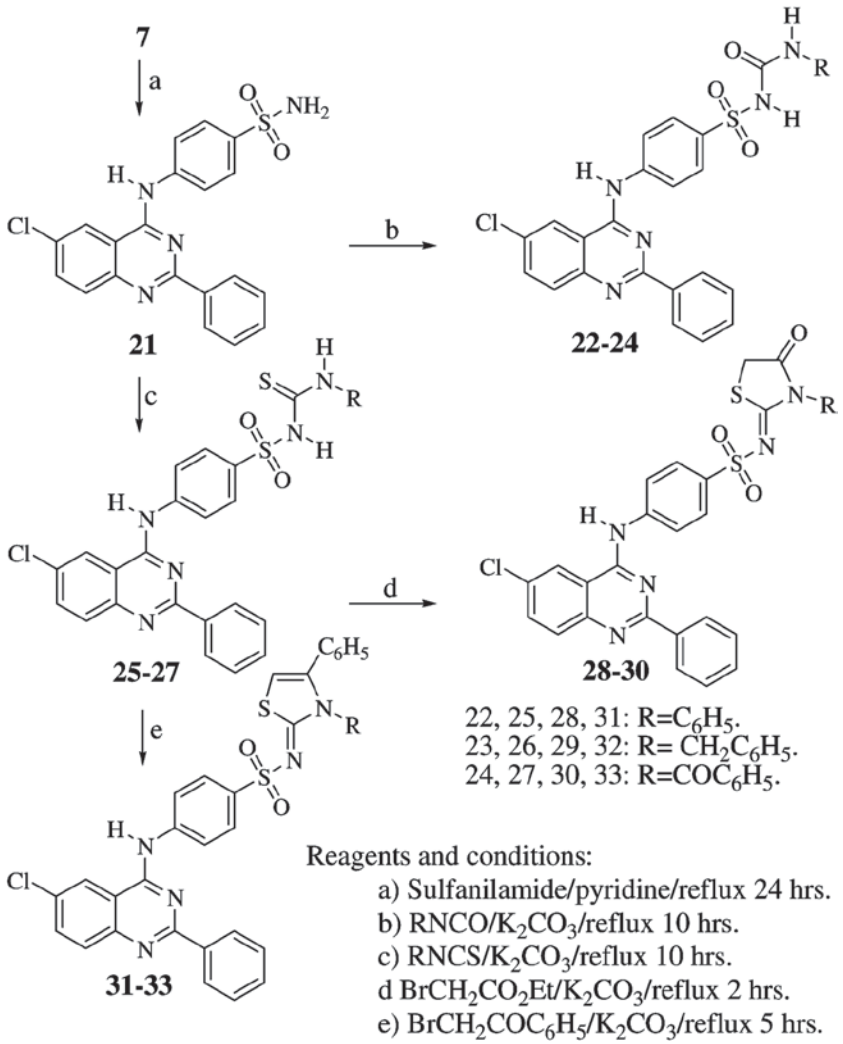
Scheme 1

Scheme 2 comprised the synthesis of *3H*-quinazolin-4-one **6**, which on chlorination using a mixture of phosphorus oxychloride and phosphorus pentachloride afforded the 4-chloroquinazoline derivative **7**. The first target sulfonamide derivative **8** was obtained by allowing the 3*H*-benzoxazin-4-one derivative **3** to condense with sulfanilamide.

The substituted sulfonylureas **9–11** were successfully obtained by condensing the sulfonamide **8** with the appropriate isocyanate in the presence of anhydrous potassium carbonate as a mild basic catalyst. The IR spectra of compounds **9–11** were characterized by a urea carbonyl band in addition to that due to the quinazoline at around 1680 cm^{-1}. Similarly, the sulfonylthiourea derivatives **12–14** were obtained by condensing compound **8** with the corresponding isothiocyanate. The IR spectra of these compounds revealed a characteristic C=S band at 1140 cm^{-1}. The latter compounds, in turn, were further utilized for the preparation of some thiazole ring systems. Thus, cyclization of the sulfonylthioureido



Scheme 2



Scheme 3

derivatives **12–14** with ethyl 2-bromoacetate in the presence of anhydrous sodium acetate afforded the corresponding thiazolidin-4-ones **15–17**. The IR spectra of these compounds were characterized by two carbonyl bands, whereas, their ^{1}H NMR spectra revealed a new singlet at around δ 4.0 ppm attributed to the oxothiazolidine C-5 two protons. Similarly, reacting the same derivatives **12–14** with phenacyl bromide under similar reaction conditions resulted in the formation of 1,3-thiazolinones **18–20**. Their IR spectra showed one C=O band at around 1685 cm^{-1} and the ^{1}H NMR spectra showed a new singlet at around δ 6.30 ppm due to the 1,3-thiazoline C-5 proton.

In the same vein, Scheme 3 represents condensation of compound **7** with sulfanilamide to afford the corresponding N4-(2-phenyl-6-chloroquinazolin-4-yl)-sulfanilamide **21**, which was also converted to the corresponding substituted sulfonylureas **22–24** and sulfonylthioureas **25–27** by condensation with the appropriate isocyanate or isothiocyanate. The thiourea derivatives were also condensed with ethyl 2-bromoacetate in the presence of anhydrous sodium acetate to afford the corresponding thiazolidin-4-ones **28–30**. Similarly, the 1,3-thiazolines **31–33** were obtained by reacting derivatives **25–27** with phenacyl bromide under alkaline conditions.

Pharmacology

Based on the results assembled in Table 1, it can be generally seen that the quinazoline derivatives **19–33**, with the benzene sulfonamide moiety at position 4, were more active than those derivatives **8**–**18** with that moiety at position 3 of the quinazoline core. Also, compounds **8** and **21** with free SO_2NH_2 group were more effective antitumor agents than their substituted derivatives. In addition, the urea derivatives proved to be more active than the thioisosteres and the 1,3-thiazolidinone derivatives **15–17** and **28–30** were more potent antitumor agents than theirthiazoline congeners **18–20** and **31–33**.

Concerning the spectrum of activity, two compounds **8** and **21** exhibited remarkable anti-proliferative activity. In our study, they showed activity comparable to that of the standard drug (with IC_{50} of 0.012 μM) against the four selected cell lines. Free sulfonamide group was a common

Table 1. IC50 of synthesized compounds against cancer cell lines.

Compound No.	IC_{50} (μM)[a]			
Cell line	MCF-7	HeLa	HepG2	HCT-8
8	0.012	0.012	0.012	0.012
9	0.035	0.040	0.062	0.033
10	0.013	0.015	0.032	0.012
11	0.019	0.017	0.050	0.017
12	0.036	0.032	0.039	0.037
13	0.038	0.039	0.039	0.035
14	0.037	0.035	0.032	0.038
15	0.019	0.029	0.031	0.028
16	0.013	0.013	0.022	0.018
17	0.013	0.020	0.050	0.015
18	0.034	0.037	0.036	0.039
19	0.037	0.035	0.036	0.033
20	0.035	0.033	0.037	0.038
21	0.012	0.012	0.012	0.012
22	0.013	0.012	0.017	0.013
23	0.015	0.013	0.013	0.014
24	0.032	0.033	0.051	0.017
25	0.031	0.037	0.034	0.037
26	0.035	0.035	0.035	0.038
27	0.035	0.036	0.037	0.034
28	0.020	0.019	0.032	0.032
29	0.015	0.015	0.015	0.015
30	0.013	0.012	0.017	0.013
31	0.020	0.032	0.027	0.038
32	0.020	0.022	0.027	0.024
33	0.012	0.013	0.012	0.013
DOX	0.011	0.011	0.011	0.011

[a]IC_{50}, compound concentration required to inhibit tumour cell proliferation by 50%.
DOX, doxorubicin.

feature in these two compounds; such moiety was reported to play a crucial role in CA inhibition and antitumor activity[7–15]. Condensing the sulfonamide moiety with different isocyanates produced the disubstituted sulfonylureido derivatives **9–11** and **22–24** of which compounds **10**, **22** and **23** exhibited good antitumor activity against the tested cell lines, but they were less active than the parent sulfonamide derivative; compound **16** displayed moderate antitumor activity against hepatoma cancer cell line and good activity against the other cell lines, compound **17** was active against human beast as well as colon cancer cell lines and moderate activity against human cervix cell line. However, compound **24** was active only against colon cancer cell line. On the other hand, converting the sulfonamide moiety to the substituted sulfonylthioureido derivatives, **12–14** and **25–27**, led to inactive products. This observation may reflect the importance of the ureido functionality rather than the thioureido in modifying this type of compounds. Incorporating the thioureido moiety in a partly rigid structure resulted in four active compounds. Compounds **16** and **17** showed moderate activity, whereas compounds **29** and **30** retained remarkable activity comparable to that of the standard drug. Thus derivatization with isocyanate led to some reduction in the extent and spectrum of activity when compared to that of the parent compound.

Conclusion

The present work led to the development of novel hybrids of quinazoline containing sulfonamide pharmacophore that exhibited remarkable antitumor activities. Four tumour cell lines, human breast cancer cell line (MCF-7), human cervix cell line (HeLa), human hepatoma cell line (HepG2) and human colon cancer cell line (HCT-8), were used to measure cytotoxic activity of the proposed quinazoline sulfonamides. All the tested compounds showed varying degrees of activity against the four selected cell lines. Few compounds were ineffective against some of the tested cell lines. Two compounds, **8** and **21**, exhibited antitumor activity comparable to that of the standard drug (doxorubicin) based on their easy synthesis. Six compounds, **16**, **22**, **23**, **29**, **30** and **33**, showed considerable activity and some were totally inactive. Finally, the remarkable activity obtained from the present series of compounds is worthy of more interest and warrants further studies to reveal the exact mechanism of action of the active compounds and to design more active and selective antitumor agents.

Experimental protocols

Chemistry

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkamp melting point apparatus (Sanyo Gallenkamp, Southborough, UK) and were uncorrected. Infra red spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra were recorded on Bruker AC-300 Ultra Shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 300 MHz for ^{1}H and 75 MHz for ^{13}C, using tetramethylsilane as internal standard. Electron impact Mass Spectra were recorded on a Shimadzu GC-MS-QP 5000 instrument (Shimadzu, Tokyo, Japan). Elemental analyses were performed, on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany), at the Microanalytical Unit, Faculty of Science, Cairo University, Cairo, Egypt, and the found results were within ±0.4% of the theoretical values.

Preparation of target compounds

Compounds **1**–**7** were prepared according to a reported procedure[16].

6-Chloro-3-(4-aminosulfonylphenyl)-2-phenyl-quinazolin-4-one **8**

A mixture of the benzoxazone **3** (1.28 g, 0.005 mol) and sulfanilamide (0.96 g, 0.0056 mol) in dry pyridine was heated under reflux for 6 h. The solvent was removed under reduced pressure and the residue was triturated with 10% hydrochloric acid, the precipitated crude product was filtered, washed with water, dried and crystallized.

8:Yield: 65%, mp: 196–198°C (ethanol). IR (cm^{-1}): 3318, 3231 (NH_2), 1670 (C=O), 1352, 1160 (SO_2).^{1}H NMR (δ, ppm): 7.47–8.16 (m, 12H, Ar-H), 8.52 (s, 2H, NH_2). ^{13}C NMR (δ, ppm): 119.6, 120.5, 123.4, 125.9, 127.5, 128.4, 129.0, 130.2, 131.8, 133.7, 135.1, 136.3, 149.5 (Ar-C), 156.0, 165.4 (C=O). MS (EI): *m/z* 413 [M^+ + 2, 13%], 411 [M^+, 38%]. Anal. Calcd for $C_{20}H_{14}ClN_3O_3S$ (411.86): C, 58.32; H, 3.43; N, 10.20; S, 7.79. Found: C, 58.49; H, 3.29; N, 9.98; S, 7.98.

***1-Substituted-3-[6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl-(4-benzenesulfonyl)]urea* 9–11**

A mixture of the sulfonamide derivative **8** (2.06 g, 0.005 mol) and anhydrous potassium carbonate (2.8 g, 0.02 mol) in *n*-butanol (25 mL) was heated under reflux with the appropriate isocyanate (0.005 mol) for 10 h. The solvent was removed under reduced pressure, and the residue was triturated with 10% hydrochloric acid, the precipitated crude product was filtered, washed with water, dried and crystallized.

1-Phenyl-3-[(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-4-benzenesulfonyl]urea **9**: Yield: 58%, mp: 136–138°C (ethanol). IR (cm^{-1}): 3317, 3202 (2NH), 3099 (CH, arom.), 1687, 1655 (2C=O), 1317, 1154 (SO_2).^{1}H NMR (δ, ppm): 7.45–8.09 (m, 17H, Ar-H), 8.95 (s, 1H, NH exchangeable), 10.53 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 118.2, 120.6, 122.0, 124.1, 126.4, 128.0, 129.2, 130.5, 132.7, 133.9, 135.5, 137.5, 144.9, 157.0, 159.3 (Ar-C), 164.4, 166.2 (2C=O). MS (EI): *m/z* 533 [M^+ + 2, 11%], 531 [M^+, 31%]. Anal. Calcd for $C_{27}H_{19}ClN_4O_4S$ (530.98): C, 61.07; H, 3.61; N, 10.55; S, 6.04. Found: C, 60.93; H, 3.40; N, 10.19; S, 5.79.

1-Benzyl-3-[(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-4-benzenesulfonyl]urea **10**: Yield: 55%, mp: 143–145°C (ethanol). IR (cm^{-1}): 3310, 3208 (2NH), 3072 (CH arom), 2986 (CH, aliph.), 1670, 1640 (2C=O), 1322, 1165 (SO_2).^{1}H NMR (δ, ppm): 4.25 (s, 2H, CH_2), 7.45–8.09 (m, 17H, Ar-H), 8.89 (s, 1H, NH exchangeable), 9.95 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 49.8 (CH_2), 120.0, 121.5, 123.1, 124.7, 126.0, 127.1, 128.2, 129.1, 130.2, 132.5, 133.8, 135.2, 136.4, 137.5, 141.5, 146.5, 156.2 (Ar-C), 161.5, 164.3 (2C=O). MS (EI): *m/z* 547 [M^+ + 2, 4%], 545 [M^+, 14%]. Anal. Calcd for $C_{28}H_{21}ClN_4O_4S$ (545.01): C, 61.71; H, 3.88, N, 10.28; S, 5.88. Found: C, 62.03; H, 4.09; N, 9.95; S, 6.19.

1-Benzoyl-3-[(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-4-benzenesulfonyl]urea **11**: Yield: 60%, mp: 159–161°C (dioxane). IR (cm^{-1}): 3307, 3222 (2NH), 3075 (CH, arom.), 1682, 1670, 1658 (3C=O), 1304, 1157 (SO_2).^{1}H NMR (δ, ppm): 7.45–8.09 (m, 17H, Ar-H), 9.94 (s, 1H, NH exchangeable), 10.58 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 118.3, 120.6, 122.5, 124.7, 126.3, 128.1, 129.0, 130.4, 132.4, 134.1, 135.6, 138.0, 145.0 (Ar-C), 155.3, 158.7, 161.5 (3C=O), 163.8 (C=N). MS (EI): *m/z* 561 [M^+ + 2, 2.7%], 559 [M^+, 8.5%]. Anal. Calcd for $C_{28}H_{19}ClN_4O_5S$ (558.99): C, 60.16; H, 3.43; N, 10.02; S, 5.74. Found: C, 59.86; H, 3.72; N, 9.70; S, 5.46.

***1-Substituted-3-[(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-4-benzene-sulfonyl]thiourea* 12–14**

To a solution of the appropriate isothiocyanate (0.011 mol) in dry acetone (5 mL) was added a mixture of the sulfonamide **8** (2.06 g, 0.005 mol) and anhydrous potassium carbonate (2.8 g, 0.02 mol) in dry acetone (25 mL), the reaction mixture was heated under reflux for 10 h. The solvent was removed under reduced pressure and the residue was triturated in 10% hydrochloric acid. The obtained crude product was filtered, washed with water, dried and crystallized.

1-Phenyl-3-[(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-4-benzene-sulfonyl]thiourea **12**: Yield: 68%, mp: 130–132°C (dioxane). IR (cm^{-1}): 3327, 3212 (2NH), 3092 (CH, arom.), 1681 (C=O), 1315, 1149 (SO_2), 1147 (C=S). ^{1}H NMR (δ, ppm): 7.43–8.12 (m, 17H, Ar-H), 8.93 (s, 1H, NH exchangeable), 10.70 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 119.9, 120.2, 121.6, 123.0, 125.3, 127.5, 128.3, 129.9, 132.6, 134.0, 135.6, 137.8, 145.3, 156.7 (Ar-C), 163.8 (C=O), 174.0 (C=S). MS (EI): *m/z* 549 [M^+ + 2, 12.4%], 547 [M^+, 37.5%]. Anal. Calcd for $C_{27}H_{19}ClN_4O_3S_2$ (547.05): C, 59.28; H, 3.50; N, 10.24; S, 11.72. Found: C, 58.93; H, 3.74; N, 9.92; S, 12.01.

1-Benzyl-3-[(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-4-benzene-sulfonyl]thiourea **13**: Yield: 65%, mp: 139–141°C (ethanol). IR (cm^{-1}): 3351, 3200 (2NH), 3087 (CH arom.), 2969 (CH, aliph.), 1682 (C=O), 1308, 1230 (SO_2), 1136 (C=S). ^{1}H NMR (δ, ppm): 4.39 (s, 2H, CH_2), 7.20–7.95 (m, 17H, Ar-H), 8.37 (s, 1H, NH exchangeable), 10.06 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 48.3 (CH_2), 121.1, 122.4, 125.6, 126.8, 127.4, 129.0, 130.4, 132.5, 133.9, 135.9, 140.6, 149.2 (Ar-C), 159.5 (C=O), 165.2 (C=N), 176.3 (C=S). MS (EI): *m/z* 563 [M^+ + 2, 19.6%], 561 [M^+, 60.9%]. Anal. Calcd for $C_{28}H_{21}ClN_4O_3S_2$ (561.07): C, 59.94; H, 3.77; N, 9.99; S, 11.43. Found: C, 60.28; H, 3.46; N, 10.25; S, 11.60.

1-Benzoyl-3-[(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-4-benzene-sulfonyl]thiourea **14**: Yield: 68%, mp: 153–155°C (dioxane). IR (cm^{-1}): 3321, 3199 (2NH), 3085 (CH, arom.), 1667, 1649 (2C=O), 1349, 1225 (SO_2), 1134 (C=S). ^{1}H NMR (δ, ppm): 7.35–8.11 (m, 17H, Ar-H), 8.90 (s, 1H, NH exchangeable), 10.56 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 120.6, 121.5, 122.4, 124.6, 126.1, 127.4, 129.0, 130.5, 132.8, 134.3, 135.5, 138.0, 144.9, 154.9(Ar-C), 163.0, 165.5 (C=O), 178.0 C=S). MS (EI): *m/z* 577 [M^+ + 2, 8.5%], 575 [M^+, 26.2%]. Anal. Calcd for $C_{28}H_{19}ClN_4O_4S_2$ (575.06): C, 58.48; H, 3.33; N, 9.74; S, 11.15. Found: C, 58.14; H, 3.05; N, 9.43; S, 10.81.

***2-[4-(6-Chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenylsulfonylimino]-3-substituted-thiazolidin-4-one* 15–17**

To a solution of the appropriate sulfonylthioureido derivatives **12–14** (0.005 mol) in *n*-butanol (20 mL), ethyl bromoacetate (1 g, 0.006 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) were added and the reaction

mixture was heated under reflux for 2 h. The mixture was then poured into ice-cold water (30 mL), and the solid product thus formed was filtered, washed with water, dried and crystallized.

2-[4-(6-Chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenylsulfonylimino]-3-phenyl-thiazolidin-4-one **15**: Yield: 66%, mp: 155–157°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3088 (CH arom.), 2985 (CH aliph.), 1687, 1654 (2C=O), 1328, 1234 (SO_2). ^1H NMR (δ, ppm): 4.05 (s, 2H, thiazol-CH_2), 7.34–7.95 (m, 17H, Ar-H). ^{13}C NMR (δ, ppm): 31.0 (thiazol-CH_2), 119.8, 120.0, 121.4, 122.7, 124.6, 126.8, 127.9, 129.5, 131.7, 133.8, 136.3, 137.9, 146.3, 158.0 (Ar-C), 163.9, 164.7 (2C=O). MS (EI): *m/z* 589 [M^+ + 2, 3.6%], 587 [M^+, 11.0%]. Anal. Calcd for $C_{29}H_{19}ClN_4O_4S_2$ (587.07): C, 59.33; H, 3.26; N, 9.54; S, 10.92. Found: C, 59.70; H, 3.51; N, 9.26; S, 11.28.

2-[4-(6-Chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenylsulfonylimino]-3-benzyl-thiazolidin-4-one **16**: Yield: 64%, mp: 163–165°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3088 (CH arom.), 2974 (CH aliph.), 1673, 1655 (2C=O), 1343, 1225 (SO_2). ^1H NMR (δ, ppm): 4.02 (s, 2H, thiazol-CH_2), 4.35 (s, 2H, benzyl-CH_2), 7.35–7.85 (m, 17H, Ar-H). ^{13}C NMR (δ, ppm): 30.0 (thiazol-CH_2), 38.5 (benzyl-CH_2), 120.1, 121.3, 122.4, 123.1, 124.0, 126.5, 127.7, 129.5, 132.0, 133.9, 135.8, 138.2, 147.0 (Ar-C), 155.5, 167.1 (2C=O), 169.1 (C=N). MS (EI): *m/z* 603 [M^+ + 2, 1.7%], 601 [M^+, 5.0%]. Anal. Calcd for $C_{30}H_{21}ClN_4O_4S_2$ (601.10): C, 59.94; H, 3.52; N, 9.32; S, 10.67. Found: C, 60.17; H, 3.80; N, 9.19; S, 10.98.

2-[4-(6-Chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenylsulfonylimino]-3-benzoyl-thiazolidin-4-one **17**: Yield: 66%, mp: 170–172°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3088 (CH arom.), 2974 (CH aliph.), 1698, 1671, 1655 (3C=O), 1340, 1227 (SO_2). ^1H NMR (δ, ppm): 3.94 (s, 2H, thiazol-CH_2), 7.34–7.87 (m, 17H, Ar-H). ^{13}C NMR (δ, ppm): 30.0 (thiazol-C), 120.0, 121.2, 122.1, 123.0, 124.5, 126.1, 127.6, 130.5, 132.4, 134.0, 135.6, 138.3, 146.4, 158.6 (Ar-C), 164.2, 165.8, 169.5 (3C=O), 171.4 (C=N). MS (EI): *m/z* 617 [M^+ + 2, 7.7%], 615 [M^+, 24.0%]. Anal. Calcd for $C_{30}H_{19}ClN_4O_5S_2$ (615.08): C, 58.58; H, 3.11; N, 9.11; S, 10.43. Found: C, 58.80; H, 2.82; N, 9.37; S, 10.71.

3-Substituted-4-phenyl-2-[4-(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenyl-sulfonylimino]-1,3-thiazoline **18–20**

A solution of the appropriate sulfonyl-thioureido derivatives **12**–**14** (0.005 mol) in absolute ethanol (20 mL) was refluxed with phenacyl bromide (1.1 g, 0.0055 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) for 5 h during which the solid product was partially crystallized out. The mixture was left to cool to room temperature, filtered, washed with water, dried and crystallized.

3,4-Diphenyl-2-[4-(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenyl-sulfonylimino]-1,3-thiazoline **18**: Yield: 66%, mp: 142–144°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3088 (CH arom.), 1687 (C=O), 1328, 1234 (SO_2). ^1H NMR (δ, ppm): 6.15 (s, 1H, thiazol-H), 7.34–7.95 (m, 22H, Ar-H). ^{13}C NMR (δ, ppm): 107.5 (thiazol-C), 118.6.0, 120.0, 121.0, 122.2, 123.1, 124.4, 125.9, 127.4, 129.7, 131.9, 134.1, 135.8, 139.0, 146.7, 148.0, 152.4 (Ar-C), 165.8, (C=O). MS (EI): *m/z* 649 [M^+ + 2, 5.5%], 647 [M^+, 19.4%]. Anal. Calcd for $C_{35}H_{23}ClN_4O_3S_2$ (647.17): C, 64.96; H, 3.58; N, 8.66; S, 9.91. Found: C, 65.30; H, 3.31; N, 8.25; S, 10.14.

3-Benzyl-4-phenyl-2-[4-(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenyl-sulfonylimino]-1,3-thiazoline **19**: Yield: 67%, mp: 152–154°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3086 (CH arom.), 2980 (CH aliph.), 1679 (C=O), 1351, 1225 (SO_2). ^1H NMR (δ, ppm): 3.79 (s, 2H, benzyl-CH_2), 6.24 (s, 1H, thiazol-H), 7.17–7.92 (m, 22H, Ar-H). ^{13}C NMR (δ, ppm): 45.1 (benzyl-C), 110.0 (thiazol-C), 118.7.0, 119.8, 121.0, 122.2, 123.4, 124.6, 125.9, 127.2, 129.0, 131.8, 133.5, 135.9, 138.5, 147.7, 150.8, 152.0 (Ar-C), 164.9, (C=O). MS (EI): *m/z* 663 [M^+ + 2, 11.5%], 661 [M^+, 35.8%]. Anal. Calcd for $C_{36}H_{25}ClN_4O_3S_2$ (661.19): C, 65.39; H, 3.81; N, 8.47; S, 9.70. Found: C, 65.25; H, 4.10; N, 8.34; S, 9.98.

3-Benzoyl-4-phenyl-2-[4-(6-chloro-4-oxo-2-phenylquinazolin-3(4H)-yl)-phenyl-sulfonylimino]-1,3-thiazoline **20**: Yield: 62%, mp: 168–170°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3088 (CH arom.), 1684, 1655 (2C=O), 1354, 1220 (SO_2). ^1H NMR (δ, ppm): 4.01 (s, 2H, benzyl-CH_2), 6.44 (s, 1H, thiazol-H), 7.13–7.88 (m, 22H, Ar-H). ^{13}C NMR (δ, ppm): 111.8 (thiazol-C), 121.2, 122.4, 123.5, 126.1, 126.8, 127.2, 128.0, 128.8, 129.3, 130.2, 131.8, 132.5, 133.7, 134.7, 135.3, 137.0, 139.2, 143.9, 150.0, 151.2, 153.6 (Ar-C), 161.5, 164.5 (2C=O). MS (EI): *m/z* 677 [M^+ + 2, 10.0%], 675 [M^+, 32.5%]. Anal. Calcd for $C_{36}H_{23}ClN_4O_4S_2$ (675.18): C, 64.04; H, 3.43; N, 8.30; S, 9.50. Found: C, 63.85; H, 3.73; N, 8.39; S, 9.72.

4-(6-Chloro-2-phenylquinazolin-4-yl-amino)-benzenesulfonamide **21**

A mixture of compound **7** (0.825 g, 0.003 mol) and sulfanilamide (0.516 g, 0.003 mol) in acetone (12 mL) in presence of anhydrous potassium carbonate (0.5 g) was heated under reflux for 5 h. The solvent was then evaporated under vacuum and the separated solid was filtered, washed with water, dried and crystallized from ethanol.

21: Yield: 61%, mp: 180–182°C (ethanol). IR (cm^{-1}): 3337, 3274, 3219 (NH, NH_2), 3085 (CH arom.), 1352, 1160 (SO_2).^1H NMR (δ, ppm): 7.47–8.16 (m, 12H, Ar-H), 8.95 (s, 2H, NH_2), 10.17 (s, 1H, NH). ^{13}C NMR (δ, ppm): 115.2, 116.5, 118.9, 126.8, 128.3, 129.1, 130.1, 131.0, 131.7, 132.6, 133.3, 134.0, 146.2, 148.5, 155.4, 1593.0 (Ar-C). MS (EI): *m/z* 413 [M^+ + 2, 14%], 411 [M^+, 45%]. Anal. Calcd for $C_{20}H_{15}ClN_4O_2S$ (410.88): C, 58.46; H, 3.68; N, 13.64; S, 7.80. Found: C, 58.69; H, 3.39; N, 13.79; S, 8.10.

***1-Substituted-3-[4-(6-chloro-2-phenylquinazolin-4-yl)-aminophenylsulfonyl]urea* 22–24**

A mixture of the sulfonamide derivative **8** *(2.06 g, 0.005 mol) and anhydrous potassium carbonate (2.8 g, 0.02 mol) in dry acetone (25 mL) was heated under reflux with the appropriate isocyanate (0.005 mol) for 24 h. The solvent was removed under reduced pressure and the residue was triturated with 10% hydrochloric acid, and then the precipitated crude product was filtered, washed with water, dried and crystallized.*

1-Phenyl-3-[4-(6-chloro-2-phenylquinazolin-4-yl)-aminophenylsulfonyl]-urea **22**: Yield: 58%, mp: 162–164°C (ethanol). IR (cm^{-1}): 3320, 3281, 3209 (3NH), 3075 (CH, arom.), 1670 (C=O), 1315, 1151 (SO_2). ^{1}H NMR (δ, ppm): 7.13–8.50 (m, 17H, Ar-H), 8.74 (s, 1H, NH exchangeable), 9.88 (s, 1H, NH exchangeable), 10.52 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 117.1, 117.7, 118.5, 121.9, 123.6, 126.1, 127.8, 128.0, 129.0 129.1, 129.4, 129.8, 130.2, 130.8, 131.2, 133.3, 136.0, 144.5, 147.1, 155.4, 157.2, 158.6(Ar-C), 166.2 (C=O). MS (EI): *m/z* 532 [M^+ + 2, 8.3%], 530 [M^+, 25%]. Anal. Calcd for $C_{27}H_{20}ClN_5O_3S$ (530.0): C, 61.19; H, 3.80; N, 13.21; S, 6.05. Found: C, 60.92; H, 3.45; N, 13.18; S, 6.20.

1-Benzyl-3-[4-(6-chloro-2-phenylquinazolin-4-yl)-aminophenylsulfonyl]urea **23**: Yield: 57%, mp: 143–145°C (ethanol). IR (cm^{-1}): 3310, 3238, 3200 (3NH), 3077 (CH arom.), 2965 (CH, aliph.), 1658 (C=O), 1321, 1160 (SO_2).^{1}H NMR (δ, ppm): 4.32 (s, 2H, CH_2), 7.25–8.16 (m, 17H, Ar-H), 8.50 (s, 1H, NH exchangeable), 9.64 (s, 1H, NH exchangeable), 10.40 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 48.1 (CH_2), 117.2, 117.8, 118.6, 121.5, 124.0, 126.3, 127.5, 128.2, 128.7, 129.0, 129.4, 129.8, 130.3, 130.9, 132.0, 133.5, 135.7, 142.8, 147.6, 154.8, 159.0 (Ar-C), 166.0 (C=O). MS (EI): *m/z* 546 [M^+ + 2, 5.3%], 544 [M^+, 14%]. Anal. Calcd for $C_{28}H_{22}ClN_5O_3S$ (544.02): C, 61.82; H, 4.08; N, 12.87; S, 5.89 Found: C, 61.70; H, 3.92; N, 13.04; S, 5.65.

1-Benzoyl-3-[4-(6-chloro-2-phenylquinazolin-4-yl)-aminophenylsulfonyl]-urea **24**: Yield: 60%, mp: 159–161°C (dioxane). IR (cm^{-1}): 3315, 3222, 3180 (3NH), 3065 (CH, arom.), 1674, 1641 (2C=O), 1311, 1154 (SO_2).^{1}H NMR (δ, ppm): 7.42–8.13 (m, 17H, Ar-H), 8.55 (s, 1H, NH exchangeable), 9.90 (s, 1H, NH exchangeable), 10.46 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 117.1, 117.6, 119.8, 128.0, 128.8, 129.6, 130.0, 130.6, 131.1, 131.8, 132.5, 133.0, 133.6, 134.8, 146.5, 148.2, 156.3, 158.5 (Ar-C), 161.4, 165.0 (2C=O). MS (EI): *m/z* 560 [M^+ + 2, 1.9%], 558 [M^+, 5.9%]. Anal. Calcd for $C_{28}H_{20}ClN_5O_4S$ (558.01): C, 60.27; H, 3.61; N, 12.55; S, 5.75. Found: C, 59.97; H, 3.82; N, 12.79; S, 5.93.

***1-Substituted-3-[(6-chloro-2-phenylquinazolin-3yl)-4-benzenesulfonyl]thiourea* 25–27**

A mixture of the sulfonamide derivative **8** (2.06 g, 0.005 mol) and anhydrous potassium carbonate (2.8 g, 0.02 mol) in dry acetone (25 mL) was heated under reflux with the appropriate isothiocyanate (0.005 mol) for 24 h. The solvent was removed under reduced pressure and the residue was triturated with 10% hydrochloric acid, the precipitated crude product was filtered, washed with water, dried and crystallized.

1-Phenyl-3-[(6-chloro-2-phenylquinazolin-3yl)-4-benzenesulfonyl]thiourea **25**: Yield: 58%, mp: 162–164°C (ethanol). IR (cm^{-1}): 3312, 3275, 3205 (3NH), 3071 (CH, arom.), 1316, 1147 (SO_2), 1125 (C=S). ^{1}H NMR (δ, ppm): 7.18–8.21 (m, 17H, Ar-H), 8.93 (s, 1H, NH exchangeable), 9.80 (s, 1H, NH exchangeable), 10.15 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 117.0, 117.4, 118.5, 121.2, 123.4, 126.0, 127.2, 128.3, 128.7 129.1, 129.5, 129.8, 130.3, 130.8, 131.2, 133.5, 135.2, 144.2, 146.8, 152.4, 157.5 (Ar-C), 181.0 (C=S). MS (EI): *m/z* 548 [M^+ + 2, 8.3%], 546 [M^+, 25%]. Anal. Calcd for $C_{27}H_{20}ClN_5O_2S_2$ (546.1): C, 59.39; H, 3.69; N, 12.83; S, 11.74. Found: C, 59.54; H, 3.95; N, 12.64; S,11.98.

1-Benzyl-3-[(6-chloro-2-phenylquinazolin-3yl)-4-benzenesulfonyl]thiourea **26**: Yield: 55%, mp: 143–145°C (ethanol). IR (cm^{-1}): 3325, 3251, 3201 (3NH), 3072 (CH arom.), 2963 (CH, aliph.), 1321, 1160 (SO_2), 1119 (C=S). ^{1}H NMR (δ, ppm): 4.20 (s, 2H, CH_2), 7.21–8.17 (m, 17H, Ar-H), 8.85 (s, 1H, NH exchangeable), 9.96 (s, 1H, NH exchangeable), 10.45 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 48.3 (CH_2), 117.1, 117.7, 118.5, 121.3, 123.5, 126.0, 127.2, 128.1, 128.5, 129.1, 129.5, 129.8, 130.3, 131.0, 131.9, 133.4, 135.5, 142.5, 147.0, 153.8, 157.4 (Ar-C), 161.9, 185.0 (C=S). MS (EI): *m/z* 562 [M^+ + 2, 5.3%], 560 [M^+, 14%]. Anal. Calcd for $C_{28}H_{22}ClN_5O_2S_2$ (560.1): C, 60.04; H, 3.96; N, 12.50; S, 11.45. Found: C, 59.77; H, 3.70; N, 12.25; S, 11.19.

1-Benzoyl-3-[(6-chloro-2-phenylquinazolin-3yl)-4-benzenesulfonyl]thiourea **27**: Yield: 60%, mp: 159–161°C (dioxane). IR (cm^{-1}): 3317, 3240, 3185 (3NH), 3071 (CH, arom.), 1674 (C=O), 1314, 1156 (SO_2), 1120 (C=S). ^{1}H NMR (δ, ppm): 7.42–8.36 (m, 17H, Ar-H), 8.84 (s, 1H, NH exchangeable), 9.91 (s, 1H, NH exchangeable), 10.50 (s, 1H, NH exchangeable). ^{13}C NMR (δ, ppm): 116.5, 117.3, 119.5, 128.0, 128.4, 129.6, 130.1, 130.5, 131.2, 131.8, 132.4, 133.0, 133.7, 134.7, 146.4, 148.1, 158, 162.5 (Ar-C), 166.0 (C=O), 184.0 (C=S). MS (EI): *m/z* 576 [M^+ + 2, 2.9%], 574 [M^+, 9%]. Anal. Calcd for $C_{28}H_{20}ClN_5O_3S_2$ (574.07): C, 58.58; H, 3.51; N, 12.20; S, 11.17. Found: C, 57.33; H, 3.80; N, 12.06; S, 10.95.

***2-[4-(6-Chloro-2-phenylquinazolin-4-yl)-aminophenylsulfonylimino]-3-substituted-thiazolidin-4-one* 28–30**

To a solution of the appropriate sulfonylthioureido derivatives **25–27** (0.005 mol) in *n*-butanol (20 mL), ethyl bromoacetate (1 g, 0.006 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 2 h, poured into ice-cold water (30 mL) and the separated solid was filtered, washed with water, dried and crystallized.

2-[4-(6-Chloro-2-phenylquinazolin-4-yl)-aminophenylsulfonylimino]-3-phenylthiazolidin-4-one **28**: Yield: 60%, mp: 155–157°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3327 (NH), 3089 (CH arom.), 2985 (CH aliph.), 1667 (C=O), 1325, 1231 (SO_2). ^{1}H NMR (δ, ppm): 4.17 (s, 2H, thiazol-CH_2), 7.23–7.95 (m, 17H, Ar-H). ^{13}C NMR (δ, ppm): 31.5 (thiazol-CH_2), 117.3, 117.9, 120.1, 121.9, 124.8, 128.1, 128.9, 129.3, 129.8, 130.5, 130.9, 131.8, 132.5, 134.6, 136.0, 146.8, 149.5 (Ar-C), 162.2, 167.0 (2C=N), 169.4 (C=O). MS (EI): *m/z* 588 [M^+ + 2, 4.6%], 586 [M^+, 15.0%]. Anal. Calcd for $C_{29}H_{20}ClN_5O_3S_2$ (586.08): C, 59.43; H, 3.44; N, 11.95; S, 10.94. Found: C, 59.71; H, 3.60; N, 12.18; S, 10.85.

2-[4-(6-Chloro-2-phenylquinazolin-4-yl)-aminophenylsulfonylimino]-3-benzylthiazolidin-4-one **29**: Yield: 63%, mp: 163–165°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3338 (NH), 3090 (CH arom.), 2958 (CH aliph.), 1677 (C=O), 1340, 1223 (SO_2). ^{1}H NMR (δ, ppm): 3.91 (s, 2H, thiazol-CH_2), 4.35 (s, 2H, benzyl-CH_2), 6.98–7.99 (m, 17H, Ar-H). ^{13}C NMR (δ, ppm): 29.8 (thiazol-CH_2), 39.7 (benzyl-CH_2), 117.2, 117.8, 119.5, 126.5, 127.1, 127.6, 128.5, 128.9, 129.2, 129.5, 130.5, 131.0, 131.6, 132.4, 134.7, 142.5, 145.0, 149.2 (Ar-C), 162.0, 164.2 (2C=N), 168.1 (C=O). MS (EI): *m/z* 602 [M^+ + 2, 2.7%], 600 [M^+, 7.2%]. Anal. Calcd for $C_{30}H_{22}ClN_5O_3S_2$ (600.11): C, 60.04; H, 3.70; N, 11.67; S, 10.69. Found: C, 60.15; H, 3.53 N, 11.85; S, 10.83.

2-[4-(6-Chloro-2-phenylquinazolin-4-yl)-aminophenylsulfonylimino]-3-benzoylthiazolidin-4-one **30**: Yield: 66%, mp: 170–172°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3328 (NH), 3088 (CH arom.), 1670, 1658 (2C=O), 1334, 1230 (SO_2). ^{1}H NMR (δ, ppm): 3.88 (s, 2H, thiazol-CH_2), 6.96–7.97 (m, 17H, Ar-H). ^{13}C NMR (δ, ppm): 30.2 (thiazol-C), 117.0, 117.9, 119.8, 126.3, 127.0, 127.6, 128.3, 128.8, 129.1, 129.7, 130.5, 131.1, 131.6, 132.7, 134.5, 142.3, 149.0 (Ar-C), 160.0, 162.4, 164.1 (3C=N), 169.0, 171.5 (2C=O). MS (EI): *m/z* 616 [M^+ + 2, 9.5%], 614 [M^+, 29.0%]. Anal. Calcdfor $C_{30}H_{20}ClN_5O_4S_2$ (614.09): C, 58.68; H, 3.28; N, 11.40; S, 10.44. Found: C, 58.47; H, 3.25; N, 11.61; S, 10.64.

***3-Substituted-4-phenyl-2-[4-(6-chloro-2-phenylquinazolin-4yl)-aminophenylsulfonylimino]-1,3-thiazoline* 31–33**

A solution of the appropriate sulfonyl-thioureido derivatives **12**–**14** (0.005 mol) in absolute ethanol (20 mL) was refluxed with phenacyl bromide (1.1 g, 0.006 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) for 5 h during which the solid product was partially crystallized out. The mixture was left to cool, filtered, washed with water, dried and crystallized.

3,4-Diphenyl-2-[4-(6-chloro-2-phenylquinazolin-4yl)-aminophenylsulfonylimino]-1,3-thiazoline **31**: Yield: 66%, mp: 142–144°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3319 (NH), 3088 (CH arom.), 1328, 1234 (SO_2). ^{1}H NMR (δ, ppm): 6.15 (s, 1H, thiazol-H), 7.34–7.95 (m, 22H, Ar-H). ^{13}C NMR (δ, ppm): 107.5 (thiazol-C), 118.6.0, 120.0, 121.0, 122.2, 123.1, 124.4, 125.9, 127.4, 129.7, 131.9, 134.1, 135.8, 139.0, 146.7 (Ar-C), 163.0, 167.4 (2C=N). MS (EI): *m/z* 648 [M^+ + 2, 5.5%], 646 [M^+, 19.4%]. Anal. Calcd for $C_{35}H_{24}ClN_5O_2S_2$(646.18): C, 65.06; H, 3.74; N, 10.84; S, 9.92. Found: C, 65.30; H, 3.31; N, 10.85; S, 10.14.

3-Benzyl-4-phenyl-2-[4-(6-chloro-2-phenylquinazolin-4yl)-aminophenylsulfonylimino]-1,3-thiazoline **32**: Yield: 60%, mp: 152–154°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3325 (NH), 3086 (CH arom.), 2980 (CH aliph.), 1351, 1225 (SO_2). ^{1}H NMR (δ, ppm): 3.79 (s, 2H, benzyl-CH_2), 6.24 (s, 1H, thiazol-H), 7.17–7.92 (m, 22H, Ar-H). ^{13}C NMR (δ, ppm): 45.1 (benzyl-C), 110.0 (thiazol-C), 118.7.0, 119.8, 121.0, 122.2, 123.4, 124.6, 125.9, 127.2, 129.0, 131.8, 133.5, 135.9, 138.5, 147.7 (Ar-C), 160.8, 164.0 (2C=N). MS (EI): *m/z* 662 [M^+ + 2, 11.5%], 660 [M^+, 35.8%]. Anal. Calcd for$C_{36}H_{26}ClN_5O_2S_2$ (660.21): C, 65.49; H, 3.97; N, 10.61; S, 9.71. Found: C, 65.25; H, 4.10; N, 10.34; S, 9.98.

3-Benzoyl-4-phenyl-2-[4-(6-chloro-2-phenylquinazolin-4yl)-aminophenylsulfonylimino]-1,3-thiazoline **33**: Yield: 52%, mp: 168–170°C (ethanol/toluene; 1:1). IR (cm^{-1}): 3321 (NH), 3088 (CH arom.),1678 (C=O), 1354, 1220 (SO_2). ^{1}H NMR (δ, ppm): 4.01 (s, 2H, benzyl-CH_2), 6.44 (s, 1H, thiazol-H), 7.13–7.88 (m, 22H, Ar-H). ^{13}C NMR (δ, ppm): 111.8 (thiazol-C), 121.2, 122.4, 123.5, 126.1, 126.8, 127.2, 128.0, 128.8, 129.3, 130.2, 131.8, 132.5, 133.7, 134.7, 135.3, 137.0, 139.2, 143.9, 150.0 (Ar-C),161.4, 162.6 (2C=N),164.5 (C=O). MS (EI): *m/z* 676 [M^+ + 2, 10.0%], 674 [M^+, 32.5%]. Anal. Calcd for$C_{36}H_{24}ClN_5O_3S_2$ (674.19): C, 64.13; H, 3.59; N, 10.39; S, 9.51. Found: C, 63.89; H, 3.73; N, 10.09; S, 9.72.

Pharmacology

Materials

Doxorubicin was supplied by LC Laboratories® (Woburn, MA). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO). The human hepatoma cell line (HepG2), human breast cancer cell line (MCF-7) human cervix cancer cell line (HeLa) and human colon cancer cell line (HCT-8) were obtained from the American Type Culture Collection (Rockville, MD). Tissue culture plates and flasks were purchased from Costar (Milan, Italy).

Evaluation of cellular cytotoxicity

The newly synthesized compounds **(8–33)** were initially screened at single concentration of 20 μg/mL using the colorimetric MTT (3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide) assay to test their *in vitro* cytotoxicity against HepG2, MCF-7, HeLaand HCT-8. The clinically used anticancer drug doxorubicin was used as standard for comparative purposes[17].

The stock solutions of the tested compounds were prepared in DMSO and were used for serial dilutions in culture medium. The tested cell lines were grown in RPMI-1640 medium supplemented with 10% calf serum.

For growth assays, exponentially growing cells were suspended in the above-mentioned medium at a density of 103–105 cells/well, seeded onto 96-well plates (200 μL/well), and incubated at 37°C in a humidified 5% CO_2 atmosphere for 24 h. After that, the cell medium in test wells was changed to new culture medium containing the required concentrations of the tested compounds, while the cell medium in control wells was changed to new culture medium containing an equivalent volume of solvent. After incubation at 37°C in a humidified 5% CO_2 atmosphere for 3 days, 100 μL of MTT (0.5 μg/mL) in serum-free medium was added to each well and incubated at 37°C for an additional 4 h. Then, 200 μL of DMSO was added to each well and mixed thoroughly to dissolve the resulting formazan product. The cell viability was evaluated by measuring the optical densities at 544 nm using a Microelisa Reader. The percentage of cell growth inhibition was calculated as follows:

% Inhibition = (Mean $OD_{control}$–Mean OD_{test})/Mean $OD_{control}$ × 100%, where OD is the optical density. The dose-response relationships of the compounds effecting ≥ 50% inhibition in one dose (20 μg/mL) prescreening for each cell line were measured using concentrations of 10, 0.1 and 0.01 μg/mL, and the concentration causing 50% cell growth inhibition (IC_{50}) was calculated. The results are shown in Table 1.

Declaration of interest

This work was supported by a grant from King Abdulaziz city for science and technology (KACST; Grant No.: ARP-29–265), and the National Plan of Science, Technology and Innovation (Grant No. 10-MED1188-02) King Saud University, Riyadh, Saudi Arabia.

References

1. Hasan J, Jayson GC. VEGF antagonists. Expert Opin Biol Ther 2001;1:703–718.
2. Hamby JM, Showalter HD. Small molecule inhibitors of tumor-promoted angiogenesis, including protein tyrosine kinase inhibitors. Pharmacol Ther 1999;82:169–193.
3. Aggarwal BB, Danda D, Gupta S, Gehlot P. Models for prevention and treatment of cancer: problems vs promises. Biochem Pharmacol 2009;78:1083–1094.
4. Csermely P, Agoston V, Pongor S. The efficiency of multi-target drugs: the network approach might help drug design. Trends Pharmacol Sci 2005;26:178–182.
5. Garofalo A, Goossens L, Baldeyrou B, Lemoine A, Ravez S, Six P et al. Design, synthesis, and DNA-binding of N-alkyl(anilino) quinazoline derivatives. J Med Chem 2010;53:8089–8103.
6. Park J, Ahn KS, Bae EK, Kim BS, Kim BK, Lee YY et al. Blockage of interleukin-6 signaling with 6-amino-4-quinazoline synergistically induces the inhibitory effect of bortezomib in human U266 cells. Anticancer Drugs 2008;19:777–782.
7. Scozzafava A, Owa T, Mastrolorenzo A, Supuran CT. Anticancer and antiviral sulfonamides. Curr Med Chem 2003;10:925–953.
8. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–181.
9. Neri D, Supuran CT. Interfering with pH regulation in tumours as a therapeutic strategy. Nat Rev Drug Discov 2011;10:767–777.
10. Owa T, Yoshino H, Okauchi T, Yoshimatsu K, Ozawa Y, Sugi NH et al. Discovery of novel antitumor sulfonamides targeting G1 phase of the cell cycle. J Med Chem 1999;42:3789–3799.
11. Van Kesteren C, Beijnen JH, Schellens JH. E7070: a novel synthetic sulfonamide targeting the cell cycle progression for the treatment of cancer. Anticancer Drugs 2002;13:989–997.
12. Minakuchi T, Nishimori I, Vullo D, Scozzafava A, Supuran CT. Molecular cloning, characterization, and inhibition studies of the Rv1284 beta-carbonic anhydrase from Mycobacterium tuberculosis with sulfonamides and a sulfamate. J Med Chem 2009;52:2226–2232.
13. Dubois L, Lieuwes NG, Maresca A, Thiry A, Supuran CT, Scozzafava A et al. Imaging of CA IX with fluorescent labelled sulfonamides distinguishes hypoxic and (re)-oxygenated cells in a xenograft tumour model. Radiother Oncol 2009;92:423–428.
14. Gitto R, Agnello S, Ferro S, De Luca L, Vullo D, Brynda J et al. Identification of 3,4-Dihydroisoquinoline-2(1H)-sulfonamides as potent carbonic anhydrase inhibitors: synthesis, biological evaluation, and enzyme–ligand X-ray studies. J Med Chem 2010;53:2401–2408.
15. Huang Z, Lin Z, Huang J. A novel kind of antitumour drugs using sulfonamide as parent compound. Eur J Med Chem 2001;36:863–872.
16. Alafeefy AM, Kadi AA, Al-Deeb OA, El-Tahir KE, Al-Jaber NA. Synthesis, analgesic and anti-inflammatory evaluation of some novel quinazoline derivatives. Eur J Med Chem 2010;45:4947–4952.
17. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990;82:1107–1112.