SYNTHESIS AND IN VITRO CYTOTOXICITY EVALUATION OF NEW 2-THIOXO-BENZO[g]QUINAZOLIN-4(3H)-ONE DERIVATIVES

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Abstract – Preparation of the titled 2-thioxo-benzo[g]quinazolin-4(3H)-ones (1–4) has been previously reported. In the present study, compounds (1–4) were elaborated in high and quantitative yields by simple modification on the reported synthetic route. Treatment of 2-thioxo-benzo[g]quinazolin-4(3H)-ones (1–4) with hydrazine hydrate or with different alkyl(heteroalkyl) halides afforded smoothly the target products 5, 6 or 7–28 in good and high yields. The in vitro cytotoxicity of compounds 1–28 was evaluated against colon HCT-116, hepatocellular Hep-G2, and breast MCF-7, prostate PC-3 and lung A-549 cancer cell lines, using MTT assay. The IC_{50}-values of the target compounds are recorded in $\mu$g/mL and doxorubicin used as a reference drug. The results revealed that compounds 1, 3, 7, 10, 13, 14, 15, 16, 20, 21 and 22 had significant cytotoxic effects in relation to the reference drug. The structures of compounds 1–28 were elucidated by means of $^1$H- and $^{13}$C-NMR and HREI mass spectrometry.

INTRODUCTION

Even though there are many therapeutic strategies, including chemotherapy have been developed for treatment of cancer and much progress has been made to understand its biology, cancer is still a serious
threat to human health worldwide. Design and developing of new bioactive molecules is one of the most important research areas in the field of medicinal chemistry. Many chemotherapeutic agents were examined, but some of them have been found unsuitable for therapeutic application due to their carcinogenic and mutagenic effects.\textsuperscript{1,2} Numerous research studies and several review articles have appeared in literature describing in detail the chemical and biological properties of benzoquinazolines.\textsuperscript{3-9} Many of benzoquinazolines played an important role in construction of many bioactive compounds used as thymidylate synthase inhibitors,\textsuperscript{10} antiviral,\textsuperscript{11} antiinflammatory,\textsuperscript{12} antidepressive,\textsuperscript{13} analgesic,\textsuperscript{14} antineoplastic and antimonoamine oxidase agents.\textsuperscript{15} Currently, high systemic toxicity and drug resistance accompanied with anticancer agents limit the successful findings in most cases. Consequently, a number of new strategies are being developed to make structural modifications to newly elaborated active compounds in order to improve their therapeutic indices and reduce their toxicity.

In continuation of research on benzoquinazolines chemistry and as a part of our interest in the search for active anticancer agents, we report herein the synthesis and study of the cytotoxic effects of a new series of 2-thioxo-benzo[g]quinazolin-4(3\textit{H})-one derivatives (1–28).

**RESULTS AND DISCUSSION**

Procedure for synthesis of benzo[g]quinazolines 1–4 (Scheme 1) has been reported in the literature,\textsuperscript{16,17} and simply modified by our research group to obtain the final products in highly or quantitative yields. We have used \textit{N,N}-dimethylformamide (DMF) as solvent for increasing the solubility of 3-amino-2-naphthoic acid and triethylamine as basic medium for the reaction. The structure of compounds 1-3 was characterized by NMR and HRMS analyses.

The new compounds (4–28) presented in this study retain the basic skeleton of benzo[g]quinazoline with an alkyl(aryl) substituent linked to the N-atom at position 3. Hydrazinolysis of compounds 1 and 2 in DMF under reflux condition for 24 h furnished the corresponding compounds 5 and 6 in good yields.

To prepare the benzo[g]quinazoline derivatives (7–28), each of compounds 1–3 was individually allowed to react with an appropriate alkyl(heteroalkyl) halide in the presence of K\textsubscript{2}CO\textsubscript{3} at 80 °C for 20 h in DMF (Scheme 1). The benzo[g]quinazolines 7–28 were obtained as amorphous powder of different colours in good to high yields and their structures were confirmed by NMR and HRMS spectrometry.

It is worth mentioning that all benzo[g]quinazolin-4(3\textit{H})-ones were newly synthesized except for their three 2-thioxo derivatives (1–3) and compound 22, which have been reported in the literature.\textsuperscript{16,17} The synthesis of all products was established through reporting of the physical properties and their identities were proved by the determination of their accurate molecular masses using HREI-MS analysis.
Final confirmation of the chemical structures was achieved by $^1$H- and $^{13}$C NMR spectroscopy and matching with the corresponding data of structurally related compounds in the literature. Basically, the essential building of the benzo[g]quinazoline structure was simply deduced from the three pairs of identical two signals each, in the range of $\delta$ 8.85–7.50 ppm in the $^1$H NMR spectra for all products (1–28).
The first pair was interpreted at about $\delta$ 8.80 and 8.20 in the form of two singlets, describable for H-5 and H-10 of the inner benzene ring. In addition, the other two pairs of aromatic signals were described as two broad-doublets at about $\delta$ 8.20 and 8.10 for H-6 and H-9 and two broad-triplets at about $\delta$ 7.66 and 7.55 for H-8 and H-7, respectively, of the outer benzene ring. Similar pattern was observed in case of the 2-thioxo (1–4) and 2-hydrazinyl (5,6) derivatives with slight upfield shift of all corresponding resonances. Moreover, $^1$H NMR spectra of 2-thioxo derivatives (1–4) showed a characteristic singlet of the $-\text{NH}$– proton at about $\delta$ 13.00, while in case of 2-hydrazinyl derivatives (5,6), two singlets were assigned at about $\delta$ 9.50 and 6.10, that were indicative for $-\text{NH}$– and $-\text{NH}_2$ protons of the hydrazinyl group. In $^{13}$C NMR spectra of all products, twelve resonances were observed for the basic benzoquinazoline-4-one structure in the range of about $\delta$ 175.0–111.0 ppm, including the most downfield shifted signals of C-2 and C-4 and most upfield ones C-7 and C-10, whereby all compounds could be sorted into three groups according to their $\delta$-values. In the first group i.e. 2-thioxo derivatives (1–4), thioxo and carbonyl carbons were recorded at about $\delta$ 174.0 (C-2) and 160.0 (C-4), while the most upfield carbons were observed at about 116.0 (C-7) and 111.0 (C-10). In contrast, the carbonyl-C (C-4) and hydrazinyl-C (C-2) were recorded at 158.9 and 148.0 in case of the 2-hydrazinyl derivatives (5,6), while C-7 and C-10 were interpreted at about 116.0 and 113.0, respectively. Whereas, the largest group of 2-thio-derivatives (7–28) was differentiated from previous groups by the location of their carbonyl-C (C-4) and thio-C (C-2) at about $\delta$ 161.0 and 156.0 ppm along with C-7 and C-10 at about 123.0 and 119.0, respectively. Unambiguous conclusion for all structures was finally obtained from the intrinsic splitting pattern (multiplicity and $J$-values) and $\delta$-values of $^1$H- and $^{13}$C-signals for the substituent/s at C-2 and C-3 in each case (ethyl, butyl, allyl, phenyl, cyclohexyl, piperidinoethyl, morpholinoethyl, phthalimidopropyl and benzyl or their derivatives), see experimental data. As an instance, all butyl derivatives (1, 5 & 7–15) exhibited the characteristic $^1$H resonances at about $\delta$ 4.0 (t), 1.70 (m), 1.40 (m) and 0.95 (t) for H-1" to H-4" and the corresponding $^{13}$C resonances at about $\delta$ 44.0, 30.0, 20.1 and 14.0 interpretable for C-1" to 4", respectively. Similarly, piperidinoethyl group showed five characteristic $^1$H resonances at about 3.50 (t, CH$_2$-7'), 2.70 (t, CH$_2$-8'), 2.50 (m, H-2'/6'), 1.40 (m, 4H, H-3'/5') and 1.38 (m, H-4') alongside their own $^{13}$C-signals at about $\delta$ 54.2 (CH$_2$-7'), 30.2 (CH$_2$-8'), 54.3 (C-2'/6'), 26.1 (C-3'/5') and 24.5 in products 13 and 26.

In addition, in case of the 2-S-aromatic substituted products (10–12, 18, 19, 24 and 25), the presence of certain substituents, such as $-\text{Cl}$, $-\text{CN}$ or $-\text{OMe}$, played an essential role in proving their identities based on their additive shift effects controlled by their inductive effect (I-effect) and/or resonance effect (R-effect), anisotropy and their positions that reflected through increment additive rules. Such effects would be very effective if we compare the $-\text{CN}$ increment effects in the splitting pattern and $\delta$-values of aromatic resonances of the benzyl group in both compounds 12 and 25 together with the distinct resonance of CN-carbon at about 119.0 ppm. The symmetrical position of $-\text{Cl}$ in the structures of 11, 19 and 25 resulted
in the appearance of aromatic protons in the form of a pair of two ortho-doublets each of 2H observed as an A2M2-spin coupling system at about δ 7.60 (H-3'/5') and 7.40 (H-2'/6') and proper δ-values for C-4' and C-1' at about 136.8 and 132.5, respectively. The 3-OMe substitution showed also drastic effect on the 13C-resonances of benzyl group-carbons in case of products 10 and 18, whereby C-3' was strongly downfield shifted to 159.7 (∆= + 30 ppm) and C-2' and C-4' strongly upfield shifted to 115.5 and 113.4 (∆= − 10 ppm), respectively. The success of the reaction with phthalic anhydride to produce 15, 21 and 28 was proved by the characteristic resonances of the planar symmetric structure of propylisoindoline moiety at 7.88 (m, 5'/6', 4'/7'), 3.79 (t, CH2-7'), 3.36 (t, CH2-9') and 2.14 (quint, CH2-8') together with their own 13C-resonances at δ 168.5 (C-1'/3'), 134.9 (C-3a'/7a'), 132.1 (C-5'/6'), 123.5 (C-4'/7'), 41.4 (CH2-7'), 36.5 (CH2-9') and 29.2 (CH2-8').

The in vitro cytotoxicity of compounds 1–28 was evaluated against PC-3, A-549, HCT-116, Hep-G2 and MCF-7 cells, using MTT assay.21 The obtained IC50-values of target compounds are summarized in Table 2 in comparison with those of doxorubicin. Most of the synthesized compounds showed significant cytotoxicity against all tested cell lines in relation to the reference drug. Compounds 3, 7, 9, 10, 14, 15 and 20 exhibited the highest cytotoxicity against HCT-116 (IC50 = 1.92–3.78 µg/mL); 14 and 20 against Hep-G2 and PC-3 (IC50 = 2.52/2.65 and 3.1/5.3 µg/mL, respectively); 3, 13, 20 against MCF-7 (IC50 = 4.66–6.82 µg/mL); 1–3, 5, 7, 9, 13, 14, 20, 21 and 26 against A-549 cells (IC50 = 3.1–6.22 µg/mL) in comparison to the reference drug doxorubicin (IC50 = 0.46, 0.51, 0.78, 0.46 and 0.91 µg/mL). Within this study, our results show that compounds 14 and 20 were the most active against all tested cell lines, whereas compounds 15 and 19 were inactive against MCF-7.

Depending on the results compiled in Table 2, it can be concluded that the structural modifications on the lead structures (1–3) may have had considerable impact on the cytotoxicity activity of all afforded derivatives (5–28). In particular, alkylation of 1 into 7, 9, 10, 14 and 15 showed increment of the cytotoxicity against HCT-116, and influenced positively effects on the activity profiles against Hep-G2, PC-3 and A-549 indicated by 14. Similarly, conversion of 2 into 20 demonstrated the highest activity against all cell lines. Hydrazinolysis of compounds 1 and 2 into 5 and 6, respectively did not exhibit remarkable change of their activity. These results indicate that replacement of the thioxo by a hydrazinyl group led to decrease in the lipophilicity of compounds 5 and 6, in relation to their parents. Variations in the position of substitution on the benzyl ring affected their cytotoxicity as shown in case of 10–12, where the presence of the methoxyl group in 10 showed remarkable increase in the cytotoxicity against all cell lines in comparison to the corresponding chloro- and cyano-derivatives (11,12). Furthermore, we have noticed that the type of alkyl or heteroalkyl groups play an important role in improvement of the cytotoxicity as observed in compounds 7, 14 and 20. This could be attributed to the magnitude and conformation of the alkyl or heteroalkyl substituents, which could be displayed a substantial role in the cytotoxic effects.
Moreover, all examined cell lines seemed to be sensitive to the antiproliferative activity of almost all tested compounds, and it is noteworthy to point out that the higher sensitivity of all cell lines was noticed towards compounds 14 and 20 followed by 1, 3, 7, 10, 13, 15, 16, 21 and 22.

Table 2. Cytotoxicity of the target compounds 1–28 (IC₅₀, μg/mL)

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<th>Compound</th>
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**EXPERIMENTAL**

**General**

Melting points were determined on open glass capillaries using a STUART Melting point SMP 10 apparatus and are uncorrected. NMR spectra were recorded on a Bruker AMX 500 spectrometer in DMSO-$d_6$ and are reported as $\delta$ ppm values relative to TMS at 500 and 125 MHz for $^1$H and $^{13}$C NMR, respectively. $J$-Values are recorded in Hz. HREI-MS spectra were measured on a JEOL the MStation JMS-700 system. Follow-up of the reactions and checking the purity of the compounds was made by TLC on DC-Mikrokarten polygram SIL G/UV$_{254}$, from the Macherey-Nagel Firm, Duren (Thickness: 0.25 mm).

**General procedure for preparation of compounds 1-4**

A mixture of alkyl(aryl) isothiocyanate (10.8 mmol), 3-amino-2-naphthoic acid (10 mmol) and triethylamine (12 mmol) in DMF (20 mL) was heated to reflux for 2–5 h. The mixture was cooled and poured onto ice-cold water. The solid was collected by filtration and dried.

**3-Butyl-2,3-dihydro-2-thioxobenzog[2]quinazolin-4(1H)-one (1):**\textsuperscript{16} pale yellow amorphous powder (98%); mp 272–274 °C (DMF); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 12.99 (s, 1H, H-7), 8.71 (s, 1H, H-5), 8.15 (br d, $J = 8.5$ Hz, 1H, H-6), 7.95 (br d, $J = 8.5$ Hz, 1H, H-9), 7.77 (s, 1H, H-10), 7.65 (br t, $J = 7.5$ Hz, 1H, H-8), 7.44 (br s, 2H, H-2, H-8), 7.25 (d, $J = 7.5$ Hz, 1H, H-9), 7.00 (s, 1H, H-10), 6.74 (d, $J = 7.5$ Hz, 1H, H-8), 6.65 (s, 1H, H-7), 5.75 (s, 1H, H-3).
1H, H-8), 7.52 (br t, J = 7.5 Hz, 1H, H-7), 4.44 (t, J = 7.5 Hz, 2H, H-1'), 1.70 (m, 2H, H-2'), 1.37 (m, 2H, H-3'), 0.95 (t, J = 7.5 Hz, 3H, H-4'); 13C NMR (125 MHz, DMSO-d6): δ 173.6 (C-2), 159.8 (C-4), 141.9 (C-4b), 136.6 (C-9a), 135.3 (C-5a), 130.0 (C-5), 129.9 (C-6), 129.8 (C-8), 127.7 (C-4a), 126.1 (C-9), 116.0 (C-7), 111.5 (C-10), 45.9 (C-1'), 29.2 (C-2'), 20.3 (C-3'), 14.2 (C-4'); HRMS (EI), m/z Calcd for C16H16N2OS (M)+ 284.0983, found 284.1003.

3-Allyl-2,3-dihydro-2-thioxobenzog[bi]quinazolin-4(1H)-one (2): pale brown amorphous powder (95%); mp 250–252 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 13.05 (s, 1H, -NH-), 8.71 (s, 1H, H-5), 8.14 (br d, J = 8.5 Hz, 1H, H-6), 7.96 (br d, J = 8.5 Hz, 1H, H-9), 7.78 (s, 1H, H-10), 7.65 (br t, J = 7.5 Hz, 1H, H-8), 7.52 (br t, J = 7.5 Hz, 1H, H-7), 5.96 (m, 1H, H-2'), 5.20 (dd, J = 17.5, 1.5 Hz, 1H, H-3a'), 5.10 (dd, J = 10.5, 1.5 Hz, 1H, H-3b'), 4.00 (d, J = 5 Hz, 2H, H-1'); 13C NMR (125 MHz, DMSO-d6): δ 175.6 (C-2), 160.9 (C-4), 142.0 (C-4b), 136.6 (C-9a), 134.8 (C-5a), 132.3 (C-2'), 130.1 (C-5), 129.9 (C-6), 129.8 (C-8), 127.8 (C-4a), 126.2 (C-9), 117.5 (C-3'), 115.6 (C-7), 111.6 (C-10), 46.1 (C-1'); HRMS (EI), m/z Calcd for C15H12N2OS (M)+ 268.0670, found 268.0694.

2,3-Dihydro-3-phenyl-2-thioxobenzog[bi]quinazolin-4(1H)-one (3): pale brown amorphous powder (90%) mp 285–287 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 13.14 (s, 1H, -NH-), 8.72 (s, 1H, H-5), 8.18 (br d, J = 8.5 Hz, 1H, H-6), 7.99 (br d, J = 8.5 Hz, 1H, H-9), 7.84 (s, 1H, H-10), 7.50 (m, J = 8 Hz, 3H, H-4', 2'/6'), 7.47 (m, 2H, H-3'/5'), 6.32 (br t-like, J = 7.5 Hz, 1H, H-4'); 13C NMR (125 MHz, DMSO-d6): δ 176.6 (C-2), 160.3 (C-4), 143.0 (C-1'), 139.8 (C-3'), 136.7 (C-9a), 135.8 (C-5a), 130.1 (C-5), 130.0 (C-3'/5'), 129.9 (C-6), 129.7 (C-8), 129.3 (C-4'), 128.6 (C-4a), 127.8 (C-2'/6'), 126.2 (C-9), 116.6 (C-7), 111.6 (C-10); HRMS (EI), m/z Calcd for C18H12N2OS (M)+ 304.0670, found 304.0697.

3-Cyclohexyl-2,3-dihydro-2-thioxobenzog[bi]quinazolin-4(1H)-one (4): brown amorphous powder (31%); mp 190–192 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 12.95 (s, 1H, -NH-), 8.67 (s, 1H, H-5), 8.13 (br d, J = 8.5 Hz, 1H, H-6), 7.96 (br d, J = 8.5 Hz, 1H, H-9), 7.74 (s, 1H, H-10), 7.64 (br t, J = 7.5 Hz, 1H, H-8), 7.51 (br t, J = 7.5 Hz, 1H, H-7), 3.96 (m, 1H, H-1'), 1.73 (m, 4H, H-2'/6'), 1.65 (m, 4H, H-3'/5'), 1.28 (m, 2H, H-4'); 13C NMR (125 MHz, DMSO-d6): δ 173.8 (C-2), 160.0 (C-4), 142.0 (C-4b), 136.7 (C-9a), 135.2 (C-5a), 130.0 (C-5), 129.9 (C-6), 129.8 (C-8), 127.8 (C-4a), 126.0 (C-9), 116.1 (C-7), 111.6 (C-10), 53.1 (C-1'), 32.8 (C-2'/6'), 25.7 (C-4'), 25.0 (3'/5'); HRMS (EI), m/z Calcd for C18H18N2OS (M)+ 310.1139, found 246. 310.1162.

General procedure for preparation of compounds 5 and 6

Compound 1 or 2 (1 mmol) was heated under reflux with hydrazine hydrate (10 mmol) in DMF (15 mL) for 24 h. After cooling, the precipitate was filtered off and washed with water to afford 5 or 6 as coloured pure amorphous powder.
3-Butyl-2-hydrazinylbenzo[g]quinazolin-4(3H)-one (5): pale green amorphous powder (72%); mp 206–208 °C (DMF); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 9.55 (s, 1H, -NH-), 8.66 (s, 1H, H-5), 8.39 (s, 1H, H-10), 8.27 (br d, \(J = 8.5\) Hz, 1H, H-6), 8.02 (br d, \(J = 8.5\) Hz, 1H, H-9), 7.76 (br t, \(J = 8\) Hz, 1H, H-8), 7.65 (br t, \(J = 8\) Hz, 1H, H-7), 6.21 (s, 2H, -NH\(_2\)), 4.22 (t, \(J = 7.5\) Hz, 2H, H-1'), 1.78 (m, 2H, H-2'), 1.40 (m, 2H, H-3'), 0.95 (t, \(J = 7.5\) Hz, 3H, H-4'); \(^1\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 158.9 (C-4), 148.0 (C-2), 144.6 (C-4b), 136.9 (C-9a), 135.7 (C-5a), 131.4 (C-5), 130.9 (C-6), 130.4 (C-8), 127.7 (C-4a), 127.3 (C-9), 116.3 (C-7), 113.3 (C-10), 42.9 (C-1'), 29.4 (C-2'), 20.0 (C-3'), 14.2 (C-4'); HRMS (EI), \(m/z\) Calcd for C\(_{16}\)H\(_{18}\)N\(_4\)O (M)\(^+\) 282.1481, found 282.1498.

3-Allyl-2-hydrazinylbenzo[g]quinazolin-4(3H)-one (6): white amorphous powder (70%); mp 236–238 °C (DMF); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 9.53 (s, 1H, -NH-), 8.40 (s, 1H, H-5), 8.24 (br d, \(J = 8.5\) Hz, 1H, H-6), 7.99 (br d, \(J = 8.5\) Hz, 1H, H-9), 7.75 (br t, \(J = 8\) Hz, 1H, H-8), 7.63 (br t, \(J = 8\) Hz, 1H, H-7), 6.02 (m, 1H, H-2'), 5.29 (dd, \(J = 17.5, 1.5\) Hz, 1H, H-3a'), 5.21 (dd, \(J = 10.5, 1.5\) Hz, 1H, H-3b'), 4.17 (d, \(J = 5\) Hz, 2H, H-1'); \(^1\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 158.8 (C-4), 147.8 (C-2), 144.6 (C-4b), 136.9 (C-9a), 135.6 (C-5a), 132.0 (C-2'), 131.4 (C-5), 130.4 (C-6), 129.1 (C-8), 127.6 (C-4a), 127.3 (C-9), 117.9 (C-3'), 116.1 (C-7), 113.2 (C-10), 45.1 (C-1'); HRMS (EI), \(m/z\) Calcd for C\(_{15}\)H\(_{14}\)N\(_4\)O (M)\(^+\) 266.1167, found 266.1181.

General procedure for preparation of compounds 7-28

To a solution of 1, 2 or 3 (1 mmol) in DMF (7 mL), potassium carbonate (1.4 mmol) was added portionwise over a period of 5 min at room temperature. After stirring for 10 min, an appropriate alkyl (heteroalkyl) halide (1.3 mmol) was added, and the reaction mixture was heated for 20 h at 80 °C. The mixture was then poured into ice/water, the formed precipitate was filtered off, washed with water and dried.

3-Butyl-2-(ethylthio)benzo[g]quinazolin-4(3H)-one (7): white amorphous powder (73%); mp 126–128 °C (DMF); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 8.79 (s, 1H, H-5), 8.18 (br d, \(J = 8.5\) Hz, 1H, H-6), 8.10 (s, 1H, H-10), 8.08 (br d, \(J = 8.5\) Hz, 1H, H-9), 7.65 (br t, \(J = 8\) Hz, 1H, H-8), 7.55 (br t, \(J = 8\) Hz, 1H, H-7), 4.06 (t, \(J = 7.5\) Hz, 2H, H-1''), 3.31 (q, \(J = 7.5\) Hz, 2H, H-1'), 1.70 (m, 2H, H-2''), 1.42 (t, \(J = 7.5\) Hz, 3H, H-2'), 1.39 (m, 2H, H-3''), 0.95 (t, \(J = 7.5\) Hz, 3H, H-4''); \(^1\)C NMR (125 MHz, DMSO-\(d_6\)): \(\delta\) 161.3 (C-4), 155.8 (C-2), 142.8 (C-4b), 136.8 (C-9a), 130.9 (C-5a), 129.7 (C-5), 129.0 (C-6), 128.4 (C-8), 128.1 (C-4a), 126.4 (C-9), 123.2 (C-7), 118.9 (C-10), 43.9 (C-1''), 30.3 (C-2''), 26.5 (C-1''), 20.1 (C-3''), 14.5 (C-2''), 14.1 (C-4''); HRMS (EI), \(m/z\) Calcd for C\(_{18}\)H\(_{20}\)N\(_2\)OS (M)\(^+\) 312.1296, found 312.1306.

2-(Allylthio)-3-butylbenzo[g]quinazolin-4(3H)-one (8): pale brown amorphous powder (85%); mp 119–121 °C (DMF); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 8.79 (s, 1H, H-5), 8.18 (br d, \(J = 8.5\) Hz, 1H, H-6), 8.11 (s, 1H, H-10), 8.07 (br d, \(J = 8.5\) Hz, 1H, H-9), 7.65 (br t, \(J = 8\) Hz, 1H, H-8), 7.55 (br t, \(J = 8\) Hz, 1H H-7), 6.05 (m, 1H, H-2'), 5.48 (dd, \(J = 17.5, 1.5\) Hz, 1H, H-3a'), 5.21 (dd, \(J = 10.5, 1.5\) Hz, 1H, H-3b'), 4.06
(t, J = 7.5 Hz, 2H, H-1’), 4.02 (d, J = 5 Hz, 2H, H-1’), 1.69 (m, 2H, H-2’), 1.39 (m, 2H, H-3’), 0.95 (t, J = 7.5 Hz, 3H, H-4’); 13C NMR (125 MHz, DMSO-d6): δ 161.3 (C-4), 155.2 (C-2), 142.6 (C-4b), 136.8 (C-9a), 133.5 (C-2’), 131.0 (C-5a), 129.8 (C-5), 129.1 (C-6), 128.4 (C-8), 128.1 (C-4a), 126.4 (C-9), 123.3 (C-7), 119.5 (C-3’), 118.9 (C-10), 44.1 (C-1”), 34.6 (C-1’), 30.3 (C-2”), 20.1 (C-3’), 14.0 (C-4’); HRMS (EI), m/z Calcd for C19H20N2OS (M)+ 324.1296, found 324.1306.

2-(Benzythio)-3-butylbenzog[quinazolin-4(3H)-one (9): white amorphous powder (82%); mp 138–140 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.79 (s, 1H, H-5), 8.19 (br d, J = 8 Hz, 1H, H-6), 8.17 (s, 1H, H-10), 8.09 (br d, J = 8 Hz, 1H, H-9), 7.66 (br t, J = 7.5 Hz, 1H, H-8), 7.56 (m, 3H, H-7, 2’/6’), 7.36 (t-like, J = 7.5 Hz, 2H, H-3’/5’), 7.28 (br d, J = 7.5 Hz, 1H, H-4’), 4.61 (s, 2H, -CH2-), 4.04 (t, J = 7.5 Hz, 2H, H-1’), 1.67 (m, 2H, H-2”), 1.37 (m, 2H, H-3”), 0.92 (t, J = 7.5 Hz, 3H, H-4’); 13C NMR (125 MHz, DMSO-d6): δ 161.3 (C-4), 155.5 (C-2), 142.6 (C-4b), 137.3 (C-1’), 136.8 (C-9a), 131.0 (C-5a), 129.9 (C-5), 129.8 (C-3’/5’), 129.1 (C-6), 129.0 (C-8), 128.4 (C-2’/6’), 128.2 (C-4a), 127.9 (C-4’), 126.5 (C-9), 123.3 (C-7), 118.9 (C-10), 44.1 (C-1”), 36.0 (-CH2-), 30.3 (C-2”), 20.1 (C-3”), 14.0 (C-4’); HRMS (EI), m/z Calcd for C23H22N2OS (M)+ 374.1453, found 374.1469.

3-Butyl-2-(3-methoxybenzylthio)benzog[quinazolin-4(3H)-one (10): white amorphous powder (85%); mp 116–118 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.80 (s, 1H, H-5), 8.20 (br d, J = 8.5 Hz, 1H, H-6), 8.18 (s, 1H, H-10), 8.09 (br d, J = 8.5 Hz, 1H, H-9), 7.66 (br t, J = 7.5 Hz, 1H, H-8), 7.56 (br t, J = 7.5 Hz, 1H, H-7), 7.27 (t-like, J = 8 Hz, 1H, H-5’), 7.16 (br s, 1H, H-2’), 7.13 (br d, J = 8 Hz, 1H, H-4’), 6.85 (br d, J = 8 Hz, 1H, H-6’), 4.58 (s, 2H, -CH2-), 4.05 (t, J = 7.5 Hz, 2H, H-1’), 3.74 (s, 3H, -OCH3), 1.68 (m, 2H, H-2”), 1.36 (m, 2H, H-3”), 0.93 (t, J = 7.5 Hz, 3H, H-4’); 13C NMR (125 MHz, DMSO-d6): δ 161.3 (C-4), 159.7 (C-3’), 155.5 (C-2), 142.6 (C-4b), 138.8 (C-1’), 136.8 (C-9a), 131.0 (C-5a), 130.1 (C-5’), 129.8 (C-5), 129.1 (C-6), 128.5 (C-8), 128.1 (C-4a), 126.5 (C-9), 123.3 (C-7), 122.0 (C-6’), 118.9 (C-10), 115.5 (C-4’), 113.4 (C-2’), 55.5 (-OCH3), 44.1 (C-1”), 36.0 (-CH2-), 30.3 (C-2”), 20.1 (C-3”), 14.0 (C-4’); HRMS (EI), m/z Calcd for C24H24N2O2S (M)+ 404.1558, found 404.1579.

3-Butyl-2-(4-chlorobenzylthio)benzog[quinazolin-4(3H)-one (11): pale yellow amorphous powder (74%); mp 147–149 °C, (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.80 (s, 1H, H-5), 8.20 (br d, J = 8 Hz, 1H, H-6), 8.18 (s, 1H, H-10), 8.10 (br d, J = 8 Hz, 1H, H-9), 7.65 (br t, J = 7.5 Hz, 1H, H-8), 7.60 (d, J = 8.5 Hz, 2H, H-3’/5’), 7.57 (br t, J = 7.5 Hz, 1H, H-7), 7.41 (d, J = 8.5 Hz, 2H, H-2’/6’), 4.61 (s, 2H, -CH2-), 4.05 (t, J = 7.5 Hz, 2H, H-1’), 1.67 (m, 2H, H-2”), 1.38 (m, 2H, H-3”), 0.93 (t, J = 7.5 Hz, 3H, H-4’); 13C NMR (125 MHz, DMSO-d6): δ 161.3 (C-4), 155.3 (C-2), 142.7 (C-4b), 136.8 (C-4’), 136.7 (C-9a), 132.5 (C-1’), 131.7 (C- 3’/5’), 131.1 (C-5a), 131.0 (C-5’), 129.8 (C-5), 129.1 (C-2’/6’), 128.9 (C-6), 128.5 (C-8), 128.1 (C-4a), 126.5 (C-9), 123.3 (C-7), 118.9 (C-10), 44.1 (C-1”), 35.1 (-CH2-), 30.3 (C-2”), 20.1 (C-3”), 14.0 (C-4’); HRMS (EI), m/z Calcd for C24H24ClN2OS (M)+ 408.1063, found 408.1089.

4-[(3-Butyl-3,4-dihydro-4-oxobenzog[quinazolin-2-ylthio)methyl]benzonitrile (12): white amorphous powder (87%); mp 178–180 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.79 (s, 1H, H-5),...
8.20 (br d, J = 7.5 Hz, 1H, H-6), 8.18 (s, 1H, H-10), 8.10 (br d, J = 7.5 Hz, 1H, H-9), 7.83 (d, J = 8.5 Hz, 2H, H-3'/5'), 7.79 (d, J = 8.5 Hz, 2H, H-2'/6'), 7.67 (br t, J = 7.5 Hz, 1H, H-8), 7.57 (br t, J = 7.5 Hz, 1H, H-7), 4.69 (s, 2H, -CH2-), 4.05 (t, J = 7.5 Hz, 2H, H-1''), 1.68 (m, 2H, H-2''), 1.37 (m, 2H, H-3''), 0.93 (t, J = 7.5 Hz, 3H, H-4''); 13C NMR (125 MHz, DMSO-d6): δ 161.7 (C-4), 155.5 (C-2), 143.9 (C-4b), 141.5 (C-1'), 136.8 (C-9a), 132.8 (C-3'/5'), 131.1 (C-5a), 130.8 (C-2'/6'), 129.8 (C-5), 128.9 (C-6), 128.5 (C-8), 128.1 (C-4a), 126.5 (C-9), 123.3 (C-7), 119.0 (C=C=N), 118.9 (C-10), 110.9 (C-4'), 44.1 (C-1''), 35.3 (-CH2-), 30.3 (C-2''), 20.1 (C-3''), 14.0 (C-4''); HRMS (EI), m/z Calcd for C24H21N3O (M)+ 399.1405, found 399.1434.

3-Butyl-2-[2-(piperidin-1-yl)ethylthio]benzo[g]quinazolin-4(3H)-one (13): pale brown amorphous powder (86%); mp 120–122 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.79 (s, 1H, H-5), 8.19 (br d, J = 7.5 Hz, 1H, H-6), 8.09 (br s, 2H, H-9, 10), 7.64 (br t, J = 7.5 Hz, 1H, H-8), 7.55 (br t, J = 7.5 Hz, 1H, H-7), 4.08 (t, J = 7.5 Hz, 2H, H-1''), 3.45 (t, J = 7.5 Hz, 2H, CH2-7'), 2.68 (t, J = 7.5 Hz, 2H, CH2-8'), 2.47 (m, 4H, H-2'/6'), 1.71 (m, 2H, H-2''), 1.40 (m, 4H, H-3'/5'), 1.39 (m, 4H, H-4''), 0.95 (t, J = 7.5 Hz, 3H, H-4''); 13C NMR (125 MHz, DMSO-d6): δ 161.4 (C-4), 155.3 (C-2), 143.3 (C-4b), 136.7 (C-9a), 130.9 (C-5a), 129.9 (C-5), 128.8 (C-6), 128.6 (C-8), 128.1 (C-4a), 126.5 (C-9), 123.3 (C-7), 118.8 (C-10), 54.3 (C-2'/6'), 54.2 (CH2-7'), 44.1 (C-1''), 30.3 (C-2''), 30.2 (CH2-8'), 26.1 (C-3'/5'), 24.5 (C-4'), 20.1 (C-3''), 14.0 (C-4''); HRMS (EI), m/z Calcd for C23H20N3OS (M)+ 397.2031, found 397.2049.

3-Butyl-2-(2-morphinoethylthio)benzo[g]quinazolin-4(3H)-one (14): pale yellow amorphous powder (88%); mp 125–127 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.79 (s, 1H, H-5), 8.18 (br d, J = 8 Hz, 1H, H-6), 8.09 (br d, J = 8 Hz, 1H, H-9), 8.07 (s, 1H, H-10), 7.65 (br t, J = 7.5 Hz, 1H, H-8), 7.55 (br t, J = 7.5 Hz, 1H, H-7), 4.07 (t, J = 7.5 Hz, 2H, H-1''), 3.90 (t, J = 4.0 Hz, 4H, H-3'/5'), 3.46 (t, J = 7.5 Hz, 2H, CH2-7'), 2.72 (t, J = 7.5 Hz, 2H, CH2-8'), 2.50 (t, J = 4.0 Hz, 4H, H-2'/6'), 1.70 (m, 2H, H-2''), 1.39 (m, 2H, H-3''), 0.95 (t, J = 7.5 Hz, 3H, H-4''); 13C NMR (125 MHz, DMSO-d6): δ 161.3 (C-4), 155.9 (C-2), 142.7 (C-4b), 136.8 (C-9a), 130.9 (C-5a), 129.8 (C-5), 129.0 (C-6), 128.4 (C-8), 128.1 (C-4a), 126.4 (C-9), 123.1 (C-7), 118.9 (C-10), 66.7 (C-3'/5'), 57.4 (CH2-7'), 53.6 (C-2'/6'), 44.0 (C-1''), 30.3 (C-2''), 29.1 (CH2-8'), 20.1 (C-3''), 14.1 (C-4''); HRMS (EI), m/z Calcd for C22H22N3O2S (M)+ 397.1824, found 397.1852.

2-[3-(3-Butyl-3,4-dihydro-4-oxobenzo[g]quinazolin-2-ythio)propyl]isooindoline-1,3-dione (15): white amorphous powder (90%); mp 210–212 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.77 (s, 1H, H-5), 8.17 (br d, J = 7.5 Hz, 1H, H-6), 7.88 (m, 5H, H-9, 5'/6', 4'/7'), 7.73 (s, 1H, H-10), 7.65 (br t, J = 7.5 Hz, 1H, H-8), 7.56 (br t, J = 7.5 Hz, 1H, H-7), 4.03 (t, J = 7.5 Hz, 2H, H-1''), 3.79 (t, J = 7 Hz, 2H, CH2-7'), 3.36 (t, J = 7 Hz, 2H, CH2-9'), 2.14 (quint, J = 7.2 Hz, 2H, CH2-8'), 1.67 (m, 2H, H-2''), 1.37 (m, 2H, H-3''), 0.93 (t, J = 7.5 Hz, 3H, H-4''); 13C NMR (125 MHz, DMSO-d6): δ 168.5 (C-1'/3'), 161.5 (C-4), 155.6 (C-2), 142.9 (C-4b), 136.8 (C-9a), 134.9 (C-3a'/7a'), 132.1 (C-5'/6'), 131.0 (C-5a), 129.9 (C-5), 129.2 (C-6), 128.5 (C-8), 128.1 (C-4a), 126.5 (C-9), 123.5 (C-4'/7'), 123.0 (C-7), 118.8 (C-10), 44.1 (C-1''), 41.4 (CH2-7'), 36.5 (CH2-9'), 30.3 (C-2''), 29.2 (CH2-8'), 20.1 (C-3''), 14.1 (C-4''); HRMS (EI),
m/z Calcd for C_{27}H_{25}N_{3}O_{3}S (M)^{+} 471.1617, found 471.1635.

3-Allyl-2-(ethylthio)benzo[g]quinazolin-4(3H)-one (16): pale yellow amorphous powder (88%); mp 98–100 °C (DMF); ^{1}H NMR (500 MHz, DMSO-d_{6}): δ 8.81 (s, 1H, H-5), 8.19 (br d, J = 8.5 Hz, 1H, H-6), 8.12 (s, 1H, H-10), 8.09 (br d, J = 8.5 Hz, 1H, H-9), 7.64 (br t, J = 8 Hz, 1H, H-8), 7.56 (br t, J = 8 Hz, 1H, H-7), 5.97 (m, 1H, H-2'), 5.22 (dd, J = 10.5, 1.5 Hz, 1H, H-3a''), 5.15 (dd, J = 17.5, 1.5 Hz, 1H, H-3b''), 4.73 (d, J = 4 Hz, 2H, H-1''), 3.31 (q, J = 7.5 Hz, 2H, H-1''), 1.41 (t, J = 7.5 Hz, 3H, H-2'); ^{13}C NMR (125 MHz, DMSO-d_{6}): δ 161.2 (C-4), 156.0 (C-2), 142.8 (C-4b), 136.9 (C-9a), 132.3 (C-2''), 131.0 (C-5a), 129.8 (C-5), 129.1 (C-6), 128.5 (C-8), 128.1 (C-4a), 126.4 (C-9), 123.3 (C-7), 118.9 (C-10), 117.7 (C-3''), 46.1 (C-1''), 26.5 (C-1''), 14.5 (C-2'); HRMS (EI), m/z Calcd for C_{17}H_{16}N_{2}SO (M)^{+} 296.0983, found 296.1011.

3-Allyl-2-(allylthio)benzo[g]quinazolin-4(3H)-one (17): pale yellow amorphous powder (79%); mp 120–122 °C (DMF); ^{1}H NMR (500 MHz, DMSO-d_{6}): δ 8.80 (s, 1H, H-5), 8.18 (br d, J = 8.5 Hz, 1H, H-6), 8.12 (s, 1H, H-10), 8.08 (br d, J = 8.5 Hz, 1H, H-9), 7.66 (br t, J = 8 Hz, 1H, H-8), 7.56 (br t, J = 8 Hz, 1H, H-7), 6.03 (m, 1H, H-2'), 5.95 (m, 1H, H-2''), 5.45 (dd, J = 17.5, 1.5 Hz, 1H, H-3a''), 5.22 (dd, J = 10.5, 1.5 Hz, 1H, H-3b''), 5.19 (dd, J = 10.5, 1.5 Hz, 1H, H-3b''), 4.72 (d, J = 4 Hz, 2H, H-1''), 4.00 (d, J = 7 Hz, 2H, H-1''); ^{13}C NMR (125 MHz, DMSO-d_{6}): δ 161.2 (C-4), 155.4 (C-2), 142.6 (C-4b), 136.9 (C-9a), 133.4 (C-2'), 132.3 (C-2''), 131.0 (C-5a), 129.8 (C-5), 129.1 (C-6), 128.5 (C-8), 128.1 (C-4a), 126.4 (C-9), 123.4 (C-7), 119.5 (C-3'), 118.9 (C-10), 117.7 (C-3''), 46.1 (C-1''), 34.7 (C-1''); HRMS (EI), m/z Calcd for C_{18}H_{16}N_{2}OS (M)^{+} 308.0983, found 308.0999.

3-Allyl-2-(3-methoxybenzylthio)benzo[g]quinazolin-4(3H)-one (18): white amorphous powder (81%); mp 115–117 °C (DMF); ^{1}H NMR (500 MHz, DMSO-d_{6}): δ 8.82 (s, 1H, H-5), 8.21 (s, 1H, H-10), 8.19 (br d, J = 8.5 Hz, 1H, H-6), 8.11 (br d, J = 8.5 Hz, 1H, H-9), 7.68 (br t, J = 7.5 Hz, 1H, H-8), 7.57 (br t, J = 7.5 Hz, 1H, H-7), 7.26 (t-like, J = 8 Hz, 1H, H-5'), 7.14 (br s, 1H, H-2'), 7.11 (br d, J = 8 Hz, 1H, H-4'), 6.86 (br d, J = 8 Hz, 1H, H-6'), 5.93 (m, 1H, H-2''), 5.20 (dd, J = 10.5, 1.5 Hz, 1H, H-3b''), 5.15 (dd, J = 17.5, 1.5 Hz, 1H, H-3a''), 4.72 (d, J = 5 Hz, 2H, H-1''), 4.58 (s, 2H, -CH_{2}-), 3.74 (s, 3H, -OCH_{3}); ^{13}C NMR (125 MHz, DMSO-d_{6}): δ 161.2 (C-4), 159.7 (C-3'), 155.7 (C-2), 142.6 (C-4b), 138.7 (C-1'), 136.9 (C-9a), 132.3 (C-2''), 131.0 (C-5a), 130.1 (C-5'), 129.8 (C-5), 129.2 (C-6), 128.6 (C-8), 128.2 (C-4a), 126.5 (C-9), 123.3 (C-7), 122.0 (C-6'), 118.9 (C-10), 117.8 (C-3''), 115.4 (C-4'), 113.4 (C-2'), 55.5 (-OCH_{3}), 46.1 (C-1''), 36.0 (-CH_{2}-); HRMS (EI), m/z Calcd for C_{23}H_{20}N_{2}O_{3}S (M)^{+} 388.1245, found 388.1247.

2-(4-Chlorobenzylthio)-3-allylbenzo[g]quinazolin-4(3H)-one (19): white amorphous powder (78%); mp 219–221 °C (DMF); ^{1}H NMR (500 MHz, DMSO-d_{6}): δ 8.82 (s, 1H, H-5), 8.21 (s, 1H, H-10), 8.18 (br d, J = 8 Hz, 1H, H-6), 8.09 (br d, J = 8 Hz, 1H, H-9), 7.66 (br t, J = 7.5 Hz, 1H, H-8), 7.59 (d, J = 8.5 Hz,
2H, H-3'/5'), 7.56 (br t, J = 7.5 Hz, 1H, H-7), 7.40 (d, J = 8.5 Hz, 2H, H-2'/6'), 5.93 (m, 1H, H-2"'), 5.21 (dd, J = 10.5, 1.5 Hz, 1H, H-3b"'), 5.13 (dd, J = 17.5, 1.5 Hz, 1H, H-3a"'), 4.72 (d, J = 4.5 Hz, 2H, H-1"'), 4.60 (s, 2H, -CH2-); 13C NMR (125 MHz, DMSO-d6): δ 161.6 (C-4), 155.4 (C-2), 142.5 (C-4b), 136.8 (C-4'), 136.7 (C-9a), 132.4 (C-1'), 132.2 (C-2'"'), 131.7 (C-3'/5'), 131.1 (C-5a), 131.0 (C-5'), 129.8 (C-5), 129.2 (C-2'/6'), 128.9 (C-6), 128.6 (C-8), 128.2 (C-4a), 126.5 (C-9), 123.4 (C-7), 118.8 (C-10), 117.8 (C-3'"'), 46.0 (C-1'"'), 35.1 (-CH2-); HRMS (EI), m/z Calcd for C22H17ClN2OS (M)+ 392.0750, found 392.0776.

3-Allyl-2-(2-morpholinoethylthio)benzo[g]quinazolin-4(3H)-one (20): brown amorphous powder (62%); mp 187–189 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.79 (s, 1H, H-5), 8.17 (br d, J = 8 Hz, 1H, H-6), 8.12 (br d, J = 8 Hz, 1H, H-9), 7.99 (s, 1H, H-10), 7.64 (br t, J = 7.5 Hz, 1H, H-8), 7.51 (br t, J = 7.5 Hz, 1H, H-7), 5.95 (m, 1H, H-2"'), 5.20 (dd, J = 10, 1.5 Hz, 1H, H-3b"'), 5.14 (dd, J = 17.5, 1.5 Hz, 1H, H-3a"'), 4.72 (d, J = 4 Hz, 2H, H-1"'), 3.59 (t, J = 4.0 Hz, 4H, H-3'/5'), 3.44 (t, J = 7.5 Hz, 2H, CH2-7'), 2.71 (t, J = 7.5 Hz, 2H, CH2-8'), 2.50 (t, J = 4.0 Hz, 4H, H-2'/6'); 13C NMR (125 MHz, DMSO-d6): δ 161.2 (C-4), 156.1 (C-2), 142.7 (C-4b), 136.8 (C-9a), 132.3 (C-2"'), 130.9 (C-5a), 129.9 (C-5), 129.1 (C-6), 128.5 (C-8), 128.1 (C-4a), 126.5 (C-9), 123.2 (C-7), 118.8 (C-10), 117.7 (C-3"'), 66.7 (C-3'/5'), 57.3 (CH2-7'), 53.5 (C-2'/6'), 34.7 (C-1'"'), 29.2 (CH2-8'); HRMS (EI), m/z Calcd for C21H23N3O2S (M)+ 381.1511, found 381.1535.

2-[3-(3-Allyl-3,4-dihydro-4-oxobenzo[g]quinazolin-2-ylthio)propyl]isoindoline-1,3-dione (21): pale yellow amorphous powder (71%); mp 140–142 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.75 (s, 1H, H-5), 8.16 (br d, J = 7.5 Hz, 1H, H-6), 7.86 (m, H-9, 5H, 4'-7'), 7.69 (s, 1H, H-10), 7.64 (br t, J = 7.5 Hz, 1H, H-8), 7.53 (br t, J = 7.5 Hz, 1H, H-7), 5.91 (m, 1H, H-2"'), 5.18 (dd, J = 10, 1.5 Hz, 1H, H-3b"'), 5.11 (dd, J = 17.5, 1.5 Hz, 1H, H-3a"'), 4.68 (d, J = 4 Hz, 2H, H-1"'), 3.78 (t, J = 7 Hz, 2H, CH2-7'), 3.32 (t, J = 7 Hz, 2H, CH2-9'), 2.11 (quint, J = 7.2 Hz, 2H, CH2-8'); 13C NMR (125 MHz, DMSO-d6): δ 168.6 (C-1'/3'), 161.6 (C-4), 155.4 (C-2), 143.0 (C-4b), 136.9 (C-9a), 134.8 (C-3a'/7a'), 132.2 (C-2"'), 132.1 (C-5''), 131.1 (C-5a), 129.8 (C-5), 129.1 (C-6), 128.4 (C-8), 128.1 (C-4a), 126.5 (C-9), 123.5 (C-4'/7'), 123.1 (C-7), 118.8 (C-10), 117.9 (C-3"'), 46.1 (C-1'"'), 41.4 (CH2-7'), 36.5 (CH2-9'), 29.2 (CH2-8'); HRMS (EI), m/z Calcd for C26H21N3O3S (M)+ 455.1304, found 455.1334.

2-(Ethylthio)-3-phenylbenzo[g]quinazolin-4(3H)-one (22): white amorphous powder (72%); mp 167–169 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.81 (s, 1H, H-5), 8.21 (br d, J = 8.5 Hz, 1H, H-6), 8.17 (s, 1H, H-10), 8.11 (br d, J = 8.5 Hz, 1H, H-9), 7.67 (br t, J = 8 Hz, 1H, H-8), 7.58 (m, 4H, H-7, 4", 2"/6"), 7.49 (m, 2H, H-3'/5"), 3.17 (q, J = 7.5 Hz, 2H, H-1"'), 1.32 (t, J = 7.5 Hz, 3H, H-2'); 13C NMR (125 MHz, DMSO-d6): δ 161.5 (C-4), 156.7 (C-2), 143.2 (C-4b), 136.9 (C-9a), 136.5 (C-1'"), 130.9 (C-5a), 130.2 (C-5), 129.9 (C-3'/5"), 129.8 (C-4'"), 129.5 (C-2"/6"), 129.1 (C-6), 128.6 (C-8), 128.2
(C-4a), 126.5 (C-9), 123.4 (C-7), 119.6 (C-10), 26.8 (C-1'), 14.3 (C-2'); HRMS (EI), m/z Calcd for C_{20}H_{16}N_{2}O_{5} S (M)^{+} 332.0983, found 332.0998.

2-(Allylthio)-3-phenylbenzo[g]quinazolin-4(3H)-one (23): pale yellow amorphous powder (77%); mp 145–147 °C (DMF); ¹H NMR (500 MHz, DMSO-d_{6}): δ 8.81 (s, 1H, H-5), 8.22 (br d, J = 8.5 Hz, 1H, H-6), 8.20 (s, 1H, H-10), 8.12 (br d, J = 8.5 Hz, 1H, H-9), 7.68 (br t, J = 8 Hz, 1H, H-8), 7.58 (m, 4H, H-7, 4", 2"/5"), 7.50 (m, 2H, H-3"/5"), 5.99 (m, 1H, H-2'), 5.37 (dd, J = 17.5, 1.5 Hz, 1H, H-3a'), 5.15 (dd, J = 10.5, 1.5 Hz, 1H, H-3b'), 3.88 (d, J = 7 Hz, 2H, H-1'); ¹³C NMR (125 MHz, DMSO-d_{6}): δ 161.5 (C-4), 156.1 (C-2), 143.1 (C-4b), 136.9 (C-9a), 136.4 (C-1"), 133.5 (C-2'), 131.0 (C-5a), 130.3 (C-5), 130.2 (C-3"/5"), 129.9 (C-4"), 129.8 (C-2"/6"), 129.2 (C-6), 128.7 (C-8), 128.2 (C-4a), 126.5 (C-9), 123.5 (C-7), 119.6 (C-10), 119.3 (C-3'), 35.1 (C-1'); HRMS (EI), m/z Calcd for C_{21}H_{16}N_{2}O_{5} S (M)^{+} 344.0983, found 344.0999.

3-[(3,4-Dihydro-4-oxo-3-phenylbenzo[g]quinazolin-2-ylthio)methyl]benzonitrile (24): pale yellow amorphous powder (84%); mp 193–195 °C (DMF); ¹H NMR (500 MHz, DMSO-d_{6}): δ 8.83 (s, 1H, H-5), 8.27 (s, 1H, H-10), 8.23 (br d, J = 8.5 Hz, 1H, H-6), 8.14 (br d, J = 8.5 Hz, 1H, H-9), 7.99 (br s, 1H, H-2'), 7.87 (br d, J = 8 Hz, 1H, H-6'), 7.71 (br d, J = 8 Hz, 1H, H-4'), 7.69 (br t, J = 8 Hz, 1H, H-8), 7.58 (br t, J = 8 Hz, 1H, H-7), 7.55 (m, 3H, 4", 2"/6"), 7.52 (m, 3H, 5', 3"/5"), 4.51 (s, 2H, -CH_{2}-); ¹³C NMR (125 MHz, DMSO-d_{6}): δ 161.5 (C-4), 156.0 (C-2), 142.9 (C-4b), 139.8 (C-1'), 136.9 (C-9a), 136.3 (C-1"), 134.8 (C-6'), 133.4 (C-2'), 131.5 (C-4'), 131.0 (C-5a), 130.4 (C-5), 130.2 (C-3"/5"), 130.1 (C-5'), 129.9 (C-4"), 129.8 (C-2"/6"), 129.3 (C-6), 128.7 (C-8), 128.2 (C-4a), 126.6 (C-9), 123.4 (C-7), 119.7 (C-10), 119.1 (C≡N), 111.7 (C-3'), 35.3 (-CH_{2}-); HRMS (EI), m/z Calcd for C_{26}H_{17}N_{3}O_{5} S (M)^{+} 419.1092, found 419.1119.

2-(4-Chlorobenzylthio)-3-phenylbenzo[g]quinazolin-4(3H)-one (25): pale yellow amorphous powder (81%); mp 195–197 °C (DMF); ¹H NMR (500 MHz, DMSO-d_{6}): δ 8.83 (s, 1H, H-5), 8.27 (s, 1H, H-10), 8.23 (br d, J = 8.5 Hz, 1H, H-6), 8.14 (br d, J = 8.5 Hz, 1H, H-9), 7.99. (br s, 1H, H-2'), 7.87 (br d, J = 8 Hz, 1H, H-6'), 7.71 (br d, J = 8 Hz, 1H, H-4'), 7.69 (br t, J = 8 Hz, 1H, H-8), 7.58 (br t, J = 8 Hz, 1H, H-7), 7.55 (m, 3H, 4", 2"/6"), 7.52 (m, 3H, 5', 3"/5"), 4.51 (s, 2H, -CH_{2}-); ¹³C NMR (125 MHz, DMSO-d_{6}): δ 161.3 (C-4), 156.0 (C-2), 143.1 (C-4b), 136.9 (C-9a), 136.8 (C-9a), 136.3 (C-1"), 134.8 (C-6'), 133.4 (C-2'), 131.5 (C-4'), 131.0 (C-5a), 130.4 (C-5), 130.2 (C-3"/5"), 130.1 (C-5'), 129.9 (C-4"), 129.8 (C-2"/6"), 129.3 (C-6), 128.7 (C-8), 128.2 (C-4a), 126.6 (C-9), 123.4 (C-7), 119.7 (C-10), 119.1 (C≡N), 111.7 (C-3'), 35.3 (-CH_{2}-); HRMS (EI), m/z Calcd for C_{25}H_{17}ClN_{3}OS (M)^{+} 428.0750, found 428.0769.

3-Phenyl-2-[2-(piperidin-1-yl)ethylthio]benzo[g]quinazolin-4(3H)-one (26): pale green amorphous powder (80%); mp 147–149 °C (DMF); ¹H NMR (500 MHz, DMSO-d_{6}): δ 8.81 (s, 1H, H-5), 8.21 (br d, J = 8.5 Hz, 1H, H-6), 8.15 (s, 1H, H-10), 8.13 (br d, J = 8.5 Hz, 1H, H-9), 7.68 (br t, J = 7.5 Hz, 1H, H-8), 7.58
(m, 4H, H-7, 4", 2"/6"), 7.49 (m, 2H, 3"/5"), 3.31 (t, J = 7 Hz, 2H, CH-2'H), 2.69 (t, J = 7 Hz, 2H, CH-2'H), 2.42 (m, 4H, H-2"/6"), 1.48 (m, 4H, H-3'/5"), 1.39 (m, 2H, H-4'), 13C NMR (125 MHz, DMSO-d6): δ 161.5 (C-4), 156.9 (C-2), 143.2 (C-4b), 136.9 (C-9a), 136.6 (C-1"), 130.9 (C-5a), 130.3 (C-3"/5"), 130.2 (C-4"), 129.9 (C-2"/6"), 129.3 (C-5), 129.0 (C-6), 128.6 (C-8), 128.2 (C-4a), 126.5 (C-9), 123.3 (C-7), 119.6 (C-10), 57.6 (CH-2'H), 54.3 (C-2'/6'), 29.9 (CH-2'H), 26.0 (C-3'/5"), 24.5 (C-4'); HRMS (EI), m/z Calcd for C_{25}H_{25}N_{3}O_{3}S (M)^+ 415.1718, found 415.1738.

2-(2-Morpholinoethylthio)-3-phenylbenzo[g]quinazolin-4(3H)-one (27): brown amorphous powder (72%); mp 172–174 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.81 (s, 1H, H-5), 8.20 (br d, J = 8 Hz, 1H, H-6), 8.15 (s, 1H, H-10), 8.12 (br d, J = 8 Hz, 1H, H-9), 7.67 (br t, J = 7 Hz, 1H, H-8), 7.58 (m, 2H, H-7, 4", 2"/6"), 7.49 (m, 2H, 3"/5"), 3.57 (t, J = 4.0 Hz, 4H, H-3'/5'), 3.32 (t, J = 7 Hz, 2H, CH-2'H), 2.63 (t, J = 7 Hz, 2H, CH-2'H), 2.46 (t, J = 4.0 Hz, 4H, H-2'/6'); 13C NMR (125 MHz, DMSO-d6): δ 161.5 (C-4), 156.7 (C-2), 143.1 (C-4b), 136.9 (C-9a), 136.5 (C-1"), 130.9 (C-5a), 130.6 (C-3"/5"), 130.4 (C-4"), 130.0 (C-2"/6"), 129.8 (C-5), 129.4 (C-6), 128.4 (C-8), 128.1 (C-4a), 126.5 (C-9), 123.3 (C-7), 119.6 (C-10), 56.6 (C-3'/5'), 53.5 (C-2'/6'), 29.4 (CH-2'H); HRMS (EI), m/z Calcd for C_{24}H_{23}N_{3}O_{2}S (M)^+ 417.1511, found 417.1525.

2-[3-(3,4-Dihydro-4-oxo-3-phenylbenzo[g]quinazolin-2-ylthio)propyl]isoindoline-1,3-dione (28): pale yellow amorphous powder (69%); mp 168–170 °C (DMF); 1H NMR (500 MHz, DMSO-d6): δ 8.78 (s, 1H, H-5), 8.19 (br d, J = 8 Hz, 1H, H-6), 8.15 (s, 1H, H-10), 8.12 (br d, J = 8 Hz, 1H, H-9), 7.67 (br t, J = 7 Hz, 1H, H-8), 7.58 (m, 4H, H-7, 4", 2"/6"), 7.49 (m, 2H, 3"/5"), 3.71 (t, J = 7 Hz, 2H, CH-2'H), 3.20 (t, J = 7 Hz, 2H, CH-2'H), 2.10 (quint, J = 7 Hz, 2H, CH-2'H); 13C NMR (125 MHz, DMSO-d6): δ 168.6 (C-1'/3'), 161.6 (C-4), 155.9 (C-2), 142.3 (C-4b), 136.9 (C-9a), 136.6 (C-1"), 134.8 (C-3a'/7a'), 132.4 (C-5'/6'), 130.8 (C-5a), 130.5 (C-3"/5"), 130.3 (C-4"), 129.9 (C-2"/6"), 129.7 (C-5), 129.2 (C-6), 128.5 (C-8), 128.1 (C-4a), 126.4 (C-9), 123.5 (C-7'), 118.9 (C-10), 41.5 (CH-2'H), 36.3 (CH-2'H), 29.4 (CH-2'H); HRMS (EI), m/z Calcd for C_{29}H_{21}N_{3}O_{3}S (M)^+ 491.1304, found 491.1321.

Antitumor activity assay

Human colon (HCT-116), human breast (MCF-7), human hepatocellular (Hep-G2), human prostate (PC-3) and human lung (A-549) carcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO2 and were subcultured two to three times a week. For antitumor assays, the cell lines were suspended in the medium at concentration 5x10^4 cell/well in Corning® 96-well tissue culture plates, and were incubated for 24 h. The tested compounds were then added to the 96-well plates (six replicates) to achieve eight concentrations for each compound. Six
vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubation for 24 h, the numbers of viable cells were determined using the MTT test. Briefly, the media were removed from the 96 well plate and replaced with 100 µL of fresh culture RPMI 1640 medium without phenol red then 10 µL of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) were added to each well including the untreated controls. The 96 well plates were then incubated at 37 °C and 5% CO₂ for 4 h. An 85 µL aliquot of the media was removed from the wells, and 50 µL of DMSO were added to each well and mixed thoroughly with the pipette and incubated at 37 °C for 10 min. Thereafter, the optical density was measured at 590 nm with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells. The percentage of viability was calculated as \[\frac{1-(OD_t/OD_c)}{}\times100\%\] where ODₜ is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration was plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The IC₅₀–values (the concentration required to cause toxic effects in 50% of intact cells) were estimated from graphic plots of the dose response curve for each concentration using Graphpad Prism software (San Diego, CA. USA)²¹-²³.

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