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ORIGINAL PAPER

Synthesis and biological evaluation of some amino- and sulfanyl-3*H*-quinazolin-4-one derivatives as potential anticancer agents

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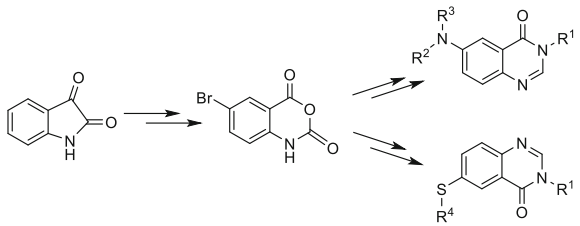
Abstract A series of 6-substituted quinazolinone derivatives were prepared by the reaction of 6-bromoquinazolinones with aryl or alkyl amines and thiols, in the presence of a $Pd(OAc)_2$/Xantphos system, under Buchwald–Hartwig-type reaction conditions. The 6-bromoquinazolinones were obtained in the three-components reaction of 5-bromoisatoic anhydride, triethyl orthoformate and an appropriate amine. Biological screening of the potential cytotoxicity of synthesized compounds on HT29 and HCT116 cell lines, as well as on the lymphocytes, showed that some derivatives of quinazolinone have significant anticancer activities. The detailed synthesis, spectroscopic data, and biological assays were reported.

Graphical abstract



Keywords Quinazolinones · Buchwald–Hartwig reaction · Palladium coupling · Anticancer activity · Sulfides

Introduction

Quinazolinone and quinazoline derivatives are a group of compounds, which arouse a great interest for medicinal chemistry, due to their diverse biological and pharmaceutical properties [1, 2]. Additionally, the quinazolinone skeleton is an important structural moiety present in many naturally occurring alkaloids [3], such as febrifugine [4, 5] isolated from an Asian plant *Dichroa febrifuga* or aniquinazolines A-D [6] and isolated from the culture of *Aspergillus nidulans*, and also it is a significant building block in the synthesis of various bioactive substances. Compounds incorporating 3*H*-quinazolin-4-one moiety were reported to possess analgesic [7], anti-inflammatory [8, 9], antibacterial [10, 11], anticonvulsant [12, 13], antifungal [14, 15], and particularly antitumor activity [16–18]. Furthermore, it was demonstrated that some of the quinazoline and quinazolinone derivatives can act as inhibitors of tubulin polymerization [19, 20] or as apoptosis inducers [21].

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Recently, we have started research on the synthesis of new derivatives of polyazanaphthalenes (quinazolinones, phthalazinones), and their benzo-analogs, with potential anticancer activity. In our previous paper [22], we described a methodology for the synthesis of *N*-substituted 6-aminobenzo[*h*]quinazolinones involving the Pd-catalyzed C–N bond formation reaction. The biological tests of 3*H*-benzo[*h*]quinazolin-4-one derivatives showed that compounds with the amino group in 6-position of lactam skeleton were highly toxic against HT29 cells, while its cytotoxicity against A549 cells and lymphocytes was significantly lower.

In the present paper, we report results of further research on the synthesis of novel derivatives of 3*H*-quinazolin-4-ones with amines and thiols, containing various substituents which would affect the activity of the target compounds. In addition, some of the newly synthesized compounds were evaluated for anticancer activity against human colon carcinoma cell lines HT29 and HCT116.

Results and discussion

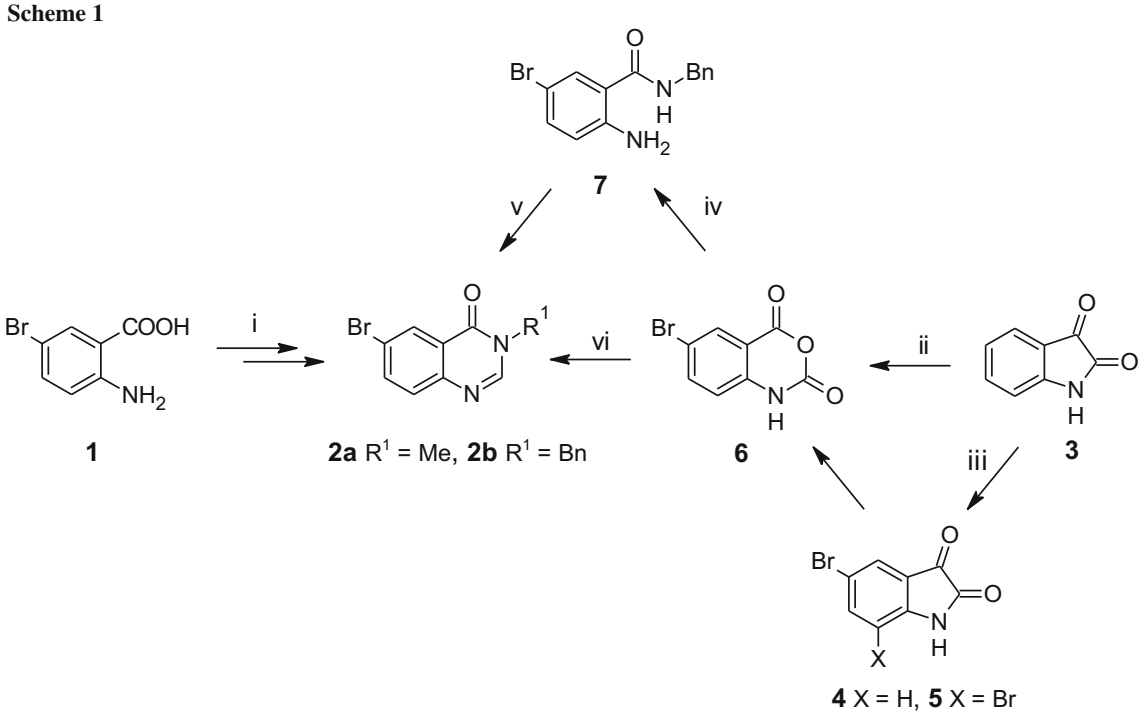
Chemistry

The target 6-amino and 6-sulfanyl derivatives of *N*-methyl- and *N*-benzylquinazolinones were synthesized from corresponding bromolactams under palladium-catalyzed Buchwald–Hartwig-type reactions.

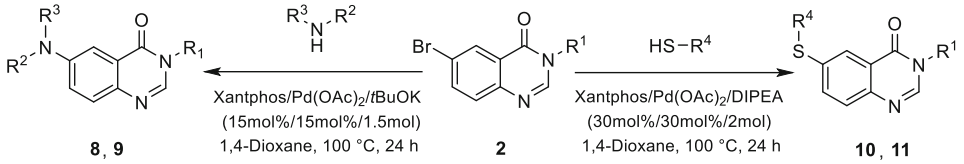
Starting bromoquinazolinones **2a**, **2b** were obtained using two approaches as shown in Scheme 1. Initially, the synthesis of quinazolinones **2a**, **2b** was carried out according to a strategy based on the Niementowski reaction and involved the condensation of **1** with formamide [22–26] and then alkylation of nitrogen atom in reaction with appropriate benzyl or methyl halides under MW irradiation conditions (Scheme 1, **1** → **2**).

Alternatively, *N*-substituted lactams **2** were prepared from corresponding isatoic anhydride (Scheme 1, **3** → **6** → **2**). The isatoic anhydrides are valuable starting substances for the synthesis of many important compounds [27–31]. During the research on the preparation of isatin derivatives, we observed that when commercially available isatin (**3**) was treated with large excess of NBS (5 equivalents) in MeCN at an ambient temperature with access to air, 5-bromoisatoic anhydride (**6**) was formed as a main product in 77 % yield. Surprisingly, 5,7-dibromoisatin (**5**) [32, 33] was not produced during this reaction. The ^{1}H, ^{13}C NMR spectra of **6** confirmed the proposed structure and were in accordance with those reported in the literature [34–36]. On the other hand, the use of 1 equivalent of NBS in the reaction with isatin, at the same reaction conditions, resulted in the formation of 5-bromoisatin (**4**) in good yield. In this case, the formation of anhydride **6** was not

Scheme 1



Scheme 2



Compound	R^1	R^2	R^3	R^4	Yield / %[a]
8a	Me	2-(morpholin-4-yl)-CH_2CH_2-	H	–	50
8b	Me	4-EtO-C_6H_4-	H	–	62
8c	Me	2-[3,4-$(MeO)_2$-C_6H_3]-CH_2CH_2-	H	–	46
8d	Me	4-Me-3-NO_2-C_6H_3-	H	–	20[b]
9a	Bn	Ph	H	–	57
9b	Bn	4-EtO-C_6H_4-	H	–	35
9c	Bn	2-[3,4-$(MeO)_2$-C_6H_3]-CH_2CH_2-	H	–	34
9d	Bn	(morpholin-4-yl)-		–	89[c]
9e	Bn	[4-(2-F-C_6H_4)piperazin-1-yl]-		–	88[c]
10a	Me	–	–	*n*-Pr	75
10b	Me	–	–	Bn	78
11	Bn	–	–	*i*-Pr	80

[a]The yield of isolated compound
[b]The NMR yield
[c]Compounds **9d**, **9e** were synthesized according to the methods described in Ref. [14]

observed. In our view, the preparation of 5-bromoisatoic anhydride **6** is a one-pot process involving in the first step bromination of **3** by *N*-bromosuccinimide, followed by ring expansion of bromoderivative **4**, with the insertion of an oxygen atom between the C-2/C-3 carbon atoms.

The mechanism of this transformation is not clear and needs more studies. However, it seems that NBS as well as oxygen from air can play important roles in the preparation process of 5-bromoisatoic anhydride from isatin. In our opinion, the presented methodology can be complementary to existing methods for the synthesis of this type of compounds [37–41].

Finally, 5-bromoisatoic anhydride was used for the preparation of bromoquinazolinones **2a**, **2b**. Treatment of anhydride **6** with benzylamine under MW conditions (320 W, 8 min) led mainly to the expected derivative **7**, which was separated in 60 % yield. Thereby, the preparation of amide **7** from **6** proved conclusively the structure of anhydride **6**. In next step, compound **7** was successfully converted into the benzylquinazolinone **2b**, in 76 % yield, in the reaction with triethyl orthoformate, in the presence of $KAl(SO_4)_2{\cdot}12H_2O$ [42, 43] (MW, 148 °C, 15 min). Based on these research results, 3-methyl derivative **2a** was synthesized in the one-pot three-component reaction between 5-bromoisatoic anhydride, triethyl orthoformate, and methylamine. Lactam **2a** was achieved in 60 % yield.

The final aminoquinazolinones **8**, **9** were successfully synthesized via palladium-catalyzed Buchwald–Hartwig cross-coupling reaction of *N*-methyl- and *N*-

benzylbromoquinazolinones **2a**, **2b** with aromatic and aliphatic amines, as depicted in Scheme 2.

Previously, we have revealed that Xantphos, $Pd(OAc)_2$ and *t*BuOK used in the ratio: 15 mol %/15 mol %/1.5 mol, in 1,4-dioxane as solvent, are an effective system for C–N bond formation [22]. The employment of the above conditions resulted in obtaining compounds **8a**–**8d** and **9a**–**9c**, in moderate to good yields (34–60 %). In all cases, the synthesis of derivatives **8**, **9** was carried out in 1,4-dioxane, under an argon and conventional heating at 100 °C, for 24 h. The achieved results, summarize in Scheme 2, demonstrated that the yields obtained were dependent on the choice of an applied amine and on the used starting lactam. Generally, *N*-methyl derivatives **8** were obtained in slightly better yields than *N*-benzyl derivatives **9**. Moreover, the arylation of 2-(3,4-dimethoxyphenyl)ethanamine with **2a** or **2b** gave corresponding products **8c** or **9c** in lower yield, in comparison with the arylation of 4-ethoxyaniline—compounds **8b**, **9b** (Scheme 2). On the other hand, unsubstituted aromatic amine, such as aniline, afforded appropriate product **9a** in higher yield than 4-ethoxyaniline (compound **9b**).

Additionally, we aimed to explore the possibility of synthesis of novel quinazolinone derivatives, containing a new C-S bond via palladium-catalyzed cross-coupling reactions [44–47] of lactams **2** with thiols (Scheme 2). Aryl sulfides are an important building block for many natural products and biologically and pharmaceutically active compounds [48–50]. Our initial studies showed that when Xantphos and $Pd(OAc)_2$ were used in the ratio 15 mol %/15 mol %, to the reaction of **2a** with 1-propanethiol, the outcomes were poor. Alkylsulfanylquinazolinone **10a** was formed in trace amounts only. It was observed that increase the amount of Xantphos/$Pd(OAc)_2$ system gave a better yield of **10a**. The optimal conditions for the arylation of alkylthiols were obtained by applying Xantphos/$Pd(OAc)_2$ system in the ratio 30 mol %/30 mol % in the presence of DIPEA and in 1,4-dioxane as solvent. The need for an increase of ligand to palladium source ratio may result from the deactivation of the palladium catalyst by thiols [51]. Based on these results, we synthesized compounds **10a**–**10b** and **11** in high yields (Scheme 2). The chemical structures of compounds **8**, **9** and **10**, **11** were determined by IR, ^{1}H NMR, ^{13}C NMR, and HRMS tests.

Anticancer activity

The anticancer activities of amino and sulfanyl derivatives **8b**, **9b**, **10a**, **10b** and additionally compounds **9d**, **9e** [22] (Scheme 2) were evaluated using two human colorectal carcinoma cell lines: HT29 and HCT116 (Table 1). The cytotoxic effect of quinazolinones **8**, **9**, **10** was also investigated on the normal human lymphocytes. The cytotoxicity was assessed by the means of an MTT test and evaluated by IC_{50} values. Compounds were studied in the concentration range from 5 to 350 μM. The obtained IC_{50} values are summarized in Table 1.

Table 1 The IC_{50} values obtained for HT29 and HCT116 cell lines and normal human lymphocytes

Compounds	IC_{50}/μM		
	HT29	HCT116	Lymphocytes
8b	29.70 ± 3.24	74.00 ± 5.24	184.67 ± 4.23
9b	33.40 ± 5.07	120.67 ± 4.62	178.00 ± 5.98
9d	59.10 ± 3.89	90.00 ± 6.17	120.67 ± 6.87
9e	50.90 ± 4.89	46.00 ± 3.91	196.67 ± 6.61
10a	29.10 ± 3.33	28.67 ± 4.40	71.33 ± 3.71
10b	45.90 ± 4.15	55.33 ± 4.58	143.33 ± 3.84

As can be seen, the most interesting results were obtained for compound **9e**, containing [4-(2-fluorophenyl)piperazin-1-yl] moiety. Compound **9e** showed significant activity towards both HT29 (IC_{50} = 50.90 μM) and HCT116 (IC_{50} = 46.00 μM) cells lines. Simultaneously, **9e** was the least toxic for lymphocytes from among tested compounds (IC_{50} = 196.67 μM). Thereby, compound **9e** demonstrated a carcinoma-specific cytotoxicity against human colon cancer cells—a strong cytotoxicity activity against both colon cancer cell lines. A similar correlation was observed for the sulfanyl derivatives of 3-methylquinazolinone **10a**, **10b**. In particular, compound **10a**, substituted by propylsulfanyl group, exhibited the most potent cytotoxicity from all tested quinazolinones: IC_{50} (HT29) = 29.10 μM and IC_{50} (HCT116) = 28.67 μM. However, it had almost a threefold lower IC_{50} value against normal cells than compound **9e**. In the case of *N*-methyl and *N*-benzyl lactams **8b**, **9b**, insertion of 4-ethoxyphenylamino group at 6-position of quinazoline skeleton resulted in potent activity towards only HT29 cell line, IC_{50} = 29.70 μM and IC_{50} = 33.40 μM, respectively. Lactams **8b**, **9b** indicated significantly less cytotoxicity against HCT116 cancer cells (IC_{50} = 74.00 - μM and IC_{50} = 120.67 μM, respectively). On the other hand, the IC_{50} values for the toxic effect on normal lymphocytes were comparable to the one obtained for **9e**.

Conclusion

In summary, we described a simple method for preparation of amino and sulfanyl derivatives of *N*-methyl- and *N*-benzylquinazolinones (compounds **8**, **9** and **10**, **11**), in satisfactory yields. The applied strategy for the C–N and C–S bond formation was based on the Buchwald–Hartwig-type reaction between *N*-substituted bromoquinazolinones **2a**, **2b** and appropriate amines or thiols, in the presence of a

$Pd(OAc)_2$/Xantphos system. The results of biological tests showed that these types of compounds could have potential applications in pharmacology and design of new anticancer agents.

Experimental

Melting points were determined on a Boetius hot-stage apparatus. ^1H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker Avance III spectrometer at 600 MHz, 150 MHz, and 565 MHz, respectively. The residual $CDCl_3$ or DMSO-d_6 signal was used for reference ($CDCl_3$ at 7.26 ppm or DMSO-d_6 at 2.54 ppm for ^1H NMR and $CDCl_3$ at 77.0 ppm or DMSO-d_6 at 39.0 ppm for ^{13}C NMR). ^{19}F NMR spectra were obtained without ^1H-decoupling. 2D homonuclear ^1H ^1H COSY spectra and heteronuclear ^1H ^{13}C COSY spectra (HSQC and HMBC) were used to assign the proton and carbon signals. IR spectra were recorded on a Nexus FT-IR spectrometer. Microwave reactions were performed in a Synthos 3000 microwave reactor from Anton Paar. LC-HRMS analyses were carried out using a liquid chromatograph (Agilent Technologies series 1200) coupled to a tandem mass spectrometer (Agilent Technologies 6538 UHD Accurate Mass Q-TOF LC/MS) equipped with a HPLC-chip cube allowing nanoelectrospray ionization of analytes. The instrument is housed in the Laboratory of Separation and Spectroscopic Method Applications, Center for Interdisciplinary Research, KUL, Lublin, Poland. Instrument control and data acquisition were performed using an Agilent Technologies Mass Hunter Acquisition module (version B.04). High-resolution mass spectra were acquired in the positive ion scan mode at $m/z = 100 - 1000$. The capillary potential was set at −1750 V and the fragmentor was set at 100 V. Mobile phase consisted of water–acetonitrile—0.1 % formic acid. A large capacity chip (0.160 mm^3, 150 mm C18) was used. Internal mass calibration was enabled, using two reference mass ions (121.0509 and 922.0098). The mass accuracy for MS scans was <1 ppm. The analytical thin-layer chromatography tests (TLC) were carried out on Supelco silica gel plates (supported on aluminum, layer thickness 200 μm) and the spots were visualized using UV lamp. The flash column chromatography purifications were performed on Fluka silica gel (Silica gel 60, 0.035–0.070 mm). All reactions with organopalladium compounds were performed under an argon atmosphere using standard Schlenk technique.

1,4-Dioxane was distilled from sodium benzophenone ketyl prior to use. Commercially available solvents and reagents, DMF, NBS, anthranilic acid, formamide, benzyl bromide, methyl iodide, isatin, and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos), were purchased from Sigma-Aldrich and were used without further purification. 3*H*-Quinazolin-4-one [22–25], 6-bromo-3*H*-quinazolin-4-one [22–25], 5,7-dibromo-1*H*-indole-2,3-dione (**5**) [52], 3-benzyl-6-(morpholin-4-yl)-3*H*-quinazolin-4-one (**9d**) [22], and 3-benzyl-6-[4-(2-fluorophenyl) piperazin-1-yl]-3*H*-quinazolin-4-one (**9e**) [22] were prepared according to known procedures.

*5-Bromo-1H-indole-2,3-dione (**4**)*

The reaction was carried out in a round-bottom flask fitted with a magnetic stirrer bar and a rubber septum with a needle as vent. To a solution of 1.00 g isatin (**3**, 6.8 mmol) in 95 cm^3 acetonitrile, a solution of 1.21 g NBS (6.8 mmol) in 15 cm^3 acetonitrile was added at temperature 0 °C. Next reaction was continued for 4 days at ambient temperature. The separated solid was collected by filtration and washed with water (2 × 10 cm^3). Then the crude product was purified by crystallization from ethanol to give pure 5-bromoisatin as an orange solid in 54 % yield. M.p.: 248–252 °C (Ref. [53] 250–254 °C).

*6-Bromo-2H-3,1-benzoxazine-2,4(1H)-dione (**6**)*

The reaction was carried out in a round-bottom flask fitted with a magnetic stirrer bar and a rubber septum with a needle as vent. To a solution of 1.00 g isatin (**3**, 6.8 mmol) in 95 cm^3 acetonitrile, a solution of 6.05 g NBS (34 mmol) in 40 cm^3 acetonitrile was added at temperature 0 °C. Next reaction was continued for 22 days at ambient temperature, with access to air. The separated solid was collected by filtration and washed with acetonitrile (2 × 10 cm^3). The pure product was obtained as a yellow solid in 77 % yield. M.p.: 245–247 °C (Ref. [35] 230–232 °C, Ref. [36] 286–288 °C).

*Preparation of N-substituted 6-bromo-3H-quinazolin-4-ones **2a**, **2b***

Reactions were carried out in an Anton Paar Synthos 3000 Microwave reactor.

Method A

3*H*-Quinazolin-4-ones **2a**, **2b** were prepared according to the already reported procedure from appropriate 6-bromo-3*H*-quinazolin-4-one [22]. Products **2a** and **2b** were purified by flash chromatography.

Method B

A mixture of 0.2 g 5-bromoisatoic anhydride (**6**, 0.826 mmol), benzylamine (0.826 mmol), and 6 cm^3 THF was charged to PTFE tube, sealed in ceramic case and placed in the rotor and then the reaction mixture was irradiated for 8 min at constant power 320 W (constant power mode) and then cooled to 35 °C. Next, 20 cm^3 water was added to the reaction mixture and stirring was continued at ambient

temperature for 15 min. The reaction mixture was extracted with chloroform (3 × 15 cm^3) and the organic layers were combined together and dried over $MgSO_4$. Evaporation of solvent gave a crude 2-amino-*N*-benzyl-5-bromobenzamide (**7**), which was purified by flash column chromatography. Next, a mixture of 0.10 g bromobenzamide **7** (0.3277 mmol), 6 cm^3 triethyl orthoformate, and 0.012 g $KAl(SO_4)_2{\cdot}12H_2O$ (0.0250 mmol) was charged to PTFE tube, sealed in ceramic case and placed in the rotor. The reaction mixture was heated to 148 °C, held for 15 min at 148 °C (constant temperature mode) and then cooled to 35 °C. After cooling, 20 cm^3 water was added to the reaction mixture and stirring was continued at ambient temperature for 15 min. Next, the reaction mixture was extracted with chloroform (3 × 15 cm^3) and the organic layers were combined together and dried over $MgSO_4$. Evaporation of solvent gave a product, **2b**, which was purified by flash column chromatography.

*2-Amino-N-benzyl-5-bromobenzamide (**7**)*
White solid, yield: 60 % (Method B), R_f (PE/AcOEt; 1/1) = 0.72; m.p.: 144–146 °C (Ref. [56, 57] 140–141 °C).

Method C: one-pot procedure

A mixture of 0.2 g 5-bromoisatoic anhydride (**6**, 0.826 mmol), $MeNH_2$—solution in THF (c = 0.11 g/cm^3, 0.826 mmol), 6 cm^3 triethyl orthoformate, and 0.03 g $KAl(SO_4)_2{\cdot}12H_2O$ (0.0632 mmol) was charged to PTFE tube, sealed in ceramic case and placed in the rotor. The reaction was heated to 148 °C, held for 15 min at 148 °C (constant temperature mode). After cooling to 35 °C, product **2a** was separated as described above—method B.

*6-Bromo-3-methyl-3H-quinazolin-4-one (**2a**)*
White solid, yield: 55 % (method A), 60 % (method C); R_f = 0.32 (DCM/MeOH 15/0.5); m.p.: 116–119 °C (Ref. [54] 338–340 °C as HBr salt); IR (KBr): 1675 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.44 (d, 1H, *J* = 2.3 Hz, 5 ArH), 8.04 (s, 1H), 7.82 (dd, 1H, *J* = 8.7, 2.3 Hz, 7 ArH), 7.57 (d, 1H, *J* = 8.7 Hz, 8 ArH), 3.59 (s, 3H, Me) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 160.5, 147.3, 147.2, 137.5, 129.5, 129.3, 123.6, 121.1, 34.3 ppm.

*3-Benzyl-6-bromo-3H-quinazolin-4-one (**2b**)*
White solid, yield: 61 % (method A), 76 % (method B); R_f = 0.48 (Hex/AcOEt 1/1); m.p.: 127–129 °C (Ref. [55] 124–126 °C).

*General procedure for the palladium-catalyzed C–N and C–S bond formations: synthesis of 6-aminoquinazolinones **8**, **9**, and 6-sulfanylquinazolinones **10**, **11***
The synthesis of 6-aminoquinazolinones **8**, **9**. The reaction was carried out under an argon atmosphere in an oven-dried resealable Schlenk flask. A resealable Schlenk flask was charged with quinazolinone **2a** or **2b** (0.19 mmol), 5 cm^3 freshly distilled dioxane, $Pd(OAc)_2$ (15 mol %), XantPhos (15 mol %), KO*t*-Bu (0.28 mmol), and the appropriate amine (0.57 mmol). The whole mixture was stirred and heated in an oil bath at 100 °C for 24 h. After this time, the reaction mixture was cooled to an ambient temperature and diluted with 5 cm^3 chloroform. The solid was removed by filtration and washed with 5 cm^3 chloroform. The filtrate was concentrated to dryness and the residue was purified by flash chromatography to give pure product.

The 6-sulfanylquinazolinones **10**, **11** were prepared according to procedure described above, using quinazolinone **2a** or **2b** (0.19 mmol), 5 cm^3 freshly distilled dioxane as solvent and $Pd(OAc)_2$ (30 mol %), XantPhos (30 mol %), DIPEA (0.38 mmol), and the appropriate thiol (0.57 mmol).

3-Methyl-6-[[2-(morpholin-4-yl)ethyl]amino]-3H-quinazolin-4-one (**8a**, $C_{15}H_{20}N_4O_2$)
Gum; yield: 50 %, R_f = 0.2 (MeCN/MeOH/AcOEt 1/0.5/1); IR (KBr): $\bar{\nu}$ = 1728, 1659 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.84 (s, 1H), 7.53 (d, 1H, *J* = 8.8 Hz, 8 ArH), 7.33 (d, 1H, *J* = 2.8 Hz, 5 ArH), 7.08 (dd, 1H, *J* = 8.8, 2.8 Hz, 7 ArH), 4.69 (s, 1H, NH), 3.77-3.73 (m, 4H, MOR), 3.57 (s, 1H, Me) 3.28 (t, 2H, *J* = 5.8 Hz, CH_2), 2.69–2.67 (m, 2H, CH_2), 2.49–2.46 (m, 4H, MOR) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.8, 147.8, 143.1, 140.7, 128.7, 123.3, 122.1, 104.8, 67.1, 57.0, 53.5, 40.0, 34.1 ppm.

6-[(4-Ethoxyphenyl)amino]-3-methyl-3H-quinazolin-4-one (**8b**, $C_{17}H_{17}N_3O_2$)
Light brown solid; yield: 62 %, R_f = 0.56 (MeCN/AcOEt 1/1); m.p.: 174–176 °C; IR (KBr): $\bar{\nu}$ = 1647 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.86 (s, 1H), 7.67 (d, 1H, *J* = 2.7 Hz, 5 ArH), 7.56 (d, 1H, *J* = 8.8 Hz, 8 ArH), 7.28–7.27 (m, 1H, 7 ArH), 7.14–7.11 (m, 2H, ArH), 6.90–6.88 (m, 2H, ArH), 5.78 (s, 1H, NH), 4.04 (q, 2H, *J* = 7.0 Hz, CH_2), 3.56 (s, 3H, Me), 1.42 (t, 3H, *J* = 7.0 Hz, Me) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.6, 155.8, 145.3, 143.8, 141.7, 134.4, 128.8, 123.6, 123.3, 122.9, 115.8, 108.6, 64.0, 34.1, 15.1 ppm.

6-[[2-(3,4-Dimethoxyphenyl)ethyl]amino]-3-methyl-3H-quinazolin-4-one (**8c**, $C_{19}H_{21}N_3O_3$)
Yellow solid; yield: 46 %, R_f = 0.4 (AcOEt/acetone 1/1); m.p.: 48–51 °C; IR (KBr): $\bar{\nu}$ = 1660 cm^{-1}; ^{1}H NMR (600, MHz, $CDCl_3$): δ = 7.83 (s, 1H), 7.51 (d, 1H, *J* = 8.8 Hz, ArH), 7.36 (d, 1H, *J* = 2.8 Hz, ArH), 6.99 (dd, 1H, *J* = 8.8, 2.8 Hz, ArH), 6.82 (d, 1H, *J* = 8.1 Hz, ArH), 6.76 (dd, 1H, *J* = 8.1, 1.9 Hz, ArH), 6.73 (d, 1H, *J* = 1.9 Hz, ArH), 4.05 (brs, 1H, NH), 3.86 (s, 6H, 2 × OMe), 3.56 (s, 3H, Me), 3.49 (t, 2H, *J* = 6.8 Hz, CH_2), 2.90 (t, 2H, *J* = 6.8 Hz, CH_2) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.7, 149.3, 148.0, 147.4, 143.1,

140.6, 131.6, 128.7, 123.4, 122.1, 120.9, 112.2, 111.7, 105.0, 56.1, 56.1, 45.1, 34.8, 34.1 ppm.

3-Benzyl-6-(phenylamino)-3H-quinazolin-4-one (**9a**, $C_{21}H_{17}N_3O$)
Beige solid; yield: 57 %, $R_f = 0.28$ (AcOEt/PE 1/1); m.p.: 172–174 °C; IR (KBr): $\overline{\nu} = 1660$ cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.97$ (s, 1H), 7.88 (d, 1H, $J = 2.7$ Hz, 5 ArH), 7.61 (d, 1H, $J = 8.8$ Hz, 8 ArH), 7.42 (dd, 1H, $J = 8.8$, 2.7 Hz, 7 ArH), 7.36–7.29 (m, 7H, PhH), 7.17–7.16 (m, 2H, PhH), 7.04–7.02 (m, 1H, PhH), 5.98 (brs, 1H, NH). 5.18 (s, 2H, CH_2) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 161.0$, 143.8, 143.4, 142.0, 141.7, 136.0, 129.7, 129.2, 128.9, 128.4, 128.1, 124.4, 123.5, 122.8, 119.5, 110.9, 49.8 ppm.

3-Benzyl-6-[(4-ethoxyphenyl)amino]-3H-quinazolin-4-one (**9b**, $C_{23}H_{21}N_3O_2$)
Light yellow solid; yield: 35 %, $R_f = 0.32$ (AcOEt/PE 10/1); m.p.: 199–200 °C; IR (KBr): $\overline{\nu} = 1663$ cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.94$ (s, 1H), 7.68 (d, 1H, $J = 2.9$ Hz, 5 ArH), 7.57 (d, 1H, $J = 8.8$ Hz, 8 ArH), 7.34–7.27 (m, 6H, ArH), 7.13–7.12 (m, 2H, ArH), 6.90–6.88 (m, 2H, ArH), 5.78 (brs, 1H, NH), 5.17 (s, 2H, CH_2), 4.03 (q, 2H, $J = 6.9$ Hz, CH_2), 1.42 (t, 3H, $J = 6.9$ Hz, Me) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 161.0$, 155.9, 145.5, 143.3, 136.1, 134.2, 129.1, 134.2, 129.1, 128.4, 128.1, 123.8, 123.5, 123.0, 115.8, 108.8, 64.0, 49.8, 15.0 ppm.

3-Benzyl-6-[[2-(3,4-dimethoxyphenyl)ethyl]amino]-3H-quinazolin-4-one (**9c**, $C_{25}H_{25}N_3O_3$)
Yellow solid; yield: 34 %, $R_f = 0.42$ (PE/DCM/MeCN 1/1/0.5); m.p.: 137–139 °C; IR (KBr): $\overline{\nu} = 1664$ cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 7.82$ (s, 1H), 7.43 (d, 1H, $J = 8.8$ Hz, 8 ArH), 7.30 (d, 1H, $J = 2.4$ Hz, 5 ArH), 7.26–7.16 (m, 6H, PhH, NH), 6.91 (dd, 1H, $J = 8.8$, 2.4 Hz, 7 ArH), 6.74 (d, 1H, $J = 8.1$ Hz, PhH), 6.69–6.63 (m, 2H, PhH), 5.10 (s, 2H, CH_2), 3.78 (s, 6H, 2 × OMe), 3.40 (t, 2H, $J = 6.8$ Hz, CH_2), 2.82 (t, 2H, $J = 6.8$ Hz, CH_2) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 161.1$, 149.2, 147.9, 147.4, 142.5, 140.2, 136.1, 131.4, 128.9, 128.6, 128.1, 127.9, 123.4, 122.1, 120.7, 112.1, 111.6, 105.2, 56.0, 55.9, 49.5, 44.9, 34.7 ppm.

3-Methyl-6-(propylsulfanyl)-3H-quinazolin-4-one (**10a**, $C_{12}H_{14}N_2OS$)
Yellow solid; yield: 75 %, $R_f = 0.64$ (acetone/DCM 1/1); m.p.: 62–64 °C; IR (KBr): $\overline{\nu} = 1667$ cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6): $\delta = 8.31$ (s, 1H), 7.95 (d, 1H, $J = 2.2$ Hz, 5 ArH), 7.73 (dd, 1H, $J = 8.6$, 2.2 Hz, 7 ArH), 7.60 (d, 1H, $J = 8.6$ Hz, 8 ArH), 3.49 (s, 3H, Me), 3.03 (t, 2H, $J = 7.2$ Hz, CH_2), 1.66–1.60 (m, 2H, CH_2), 0.99 (t, 3H, $J = 7.3$ Hz, Me) ppm; ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 197.7$, 185.5, 183.6, 173.5, 171.5, 165.5, 160.8, 159.5, 71.6, 71.2, 59.3, 50.7, 37.7 ppm.

6-(Benzylsulfanyl)-3-methyl-3H-quinazolin-4-one (**10b**, $C_{16}H_{14}N_2OS$)
Light yellow solid; yield: 78 %, $R_f = 0.64$ (AcOEt/MeCN 1/1); m.p.: 108–111 °C; IR (KBr): $\overline{\nu} = 1674$ cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 8.22$ (d, 1H, $J = 2.0$ Hz, 5 ArH), 7.98 (s, 1H), 7.62 (dd, 1H, $J = 8.5$, 2.2 Hz, 7 ArH), 7.57 (d, 1H, $J = 8.5$ Hz, 8 ArH), 7.36–7.33 (m, 2H, PhH), 7.30–7.28 (m, 2H, PhH), 7.25–7.23 (m, 1H, PhH), 4.23 (s, 2H, CH_2), 3.58 (s, 3H, Me) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 161.1$, 147.2, 146.7, 146.5, 137.5, 136.9, 136.7, 135.2, 129.0, 128.8, 128.0, 127.6, 125.6, 122.5, 38.7, 34.2 ppm.

3-Benzyl-6-(propan-2-ylsulfanyl)-3H-quinazolin-4-one (**11**, $C_{18}H_{18}N_2OS$)
Light yellow solid; yield: 80 %, $R_f = 0.60$ (AcOEt/DCM/PE 1/9/3); m.p.: 59–61 °C; IR (KBr): $\overline{\nu} = 1674$ cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = 8.27$ (d, 1H, $J = 2.1$ Hz, 5 ArH), 8.07 (s, 1H), 7.70 (dd, 1H, $J = 8.5$, 2.1 Hz, 7 ArH), 7.61 (d, 1H, $J = 8.5$ Hz, 8 ArH), 7.36–7.30 (m, 5H, PhH), 5.19 (s, 2H, CH_2), 3.56–3.51 (m, 1H, CH), 1.34 (d, 6H, $J = 6.7$ Hz, 2 × Me) ppm; ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 160.7$, 146.5, 146.1, 136.9, 136.6, 135.8, 134.6, 129.2, 128.5, 128.2, 128.0, 127.8, 124.5, 122.7, 49.9, 38.1, 31.0, 23.2 ppm.

Cells cultures

The experiments were performed with the use of HCT116 (colorectal carcinoma) and HT29 (colorectal adenocarcinoma) cancer cells (human colon cancer cells) derived from the American Type Culture Collection (ATCC; CCL-247, HTB-38) and human lymphocytes obtained from the Blood Donation Centre (Lodz, Poland).

HCT116 cells were cultured in RPMI 1640 medium (CytoGen) supplemented with 10 % FBS (Foetal Bovine Serum, CytoGen) and penicillin/streptomycin solution (1 %).

RPMI 1640 medium (CytoGen) was used for HT29 cells containing FBS (10 %), penicillin/streptomycin solution (1 %), and MEM non-essential amino acids solution (1 %). Human lymphocytes were cultured in RPMI 1640 medium complemented with inactivated FBS (15 %), penicillin/streptomycin (1 %), and mitogen PHA (1 %, phytohemagglutinin; CytoGen). Cells were cultured at 37 °C in a 5 % CO_2 humidified atmosphere. All solvents and reagents were obtained from Sigma-Aldrich.

Inhibition growth assay

Cancer cells and human lymphocytes were grown for 24 h on 96-well plates at a density of 6–8 × 10^3 cells/well and 8 × 10^5 cells/well, respectively. Then the cells were treated

with the tested compounds for 72 h. After the treatment, MTT dye dissolved in PBS was added to each plate well for 4 h. Purple crystals formed in cancer cells after the reduction of MTT were dissolved by DMSO (100 mm^3/well) after removing of the RPMI 1640 medium. In the case of lymphocytes, it was done by adding 100 mm^3 of 20 % DMF and 50 % SDS mixture to each well for 24 h. Absorbance at 595 nm was measured with a spectrophotometer PowerWave XS (BioTek Instruments, Inc.).

The cell survival effect was expressed as the IC_{50} value which is the concentration of the compound required to reduce cell survival to 50 % as compared to the negative control. The experiments were done in triplicate. All the results were presented as the mean ± SD.

MTT assay

MTT assay is a quantitative colorimetric method to determine cell proliferation after the treatment with the tested compounds. It is widely used to estimate the cytotoxic effect of chemicals on different types of cells. The assay is based on the reduction of the yellow, water-soluble tetrazolium MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] by mitochondrial enzymes of living (not dead) cells which results in the formation of an insoluble purple formazan product. Formazan crystals are solubilized with organic solvent. The amount of formazan is measured spectrophotometrically and it is directly proportional to the number of living cells.

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