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Evaluation of antitumor potential of synthesized novel 2-substituted 4-anilinoquinazolines as quinazoline-pyrrole hybrids in MCF-7 human breast cancer cell line and A-549 human lung adenocarcinoma cell lines

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Abstract

Background: A series of novel 2 substituted 4-anilinoquinazolines-pyrrole hybrids were synthesized, and cytotoxic activity were evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay.

Methods: The cell line used for the activity was MCF-7 breast cancer cell line and A459 human lung adenocarcinoma cell line. The newly quinazoline-pyrrole hybrid compounds have been synthesized from the 4-chloro-7-(3-chloropropoxy)-6-methoxy-2-phenylquinazoline derivatives. The chemical structure of the synthesized compounds has been confirmed by FTIR, ^{1}HNMR, ^{13}C NMR, and mass spectral data. The cytotoxic study was conducted using morphological study and MTT assay against adenocarcinoma and human breast cancer cell lines.

Results: The results of cytotoxic evaluation revealed that few compounds show moderate to promising activity when compared with standard doxorubicin (IC_{50} value 41.05 μM at 72 h). The synthesized compounds 7d and 7f were found effective in breast cancer cell line with IC_{50} values 40.64 μM and 44.98 μM at 72 h, respectively. The synthesized compounds 7d, 7f, 7g, and 7h were found effective in adenocarcinoma cell line with IC_{50} values of 41.05 μM, 45.54 μM, 46.93 μM, and 48.62 μM, respectively.

Conclusion: Based on the experimental evidences, we proposed structure activity relationship to provide significant information for the design and development of further potent anticancer agents.

Keywords: Quinazolines, Pyrrole, Cytotoxic, Breast cancer, Adenocarcinoma

Background

The heterocyclic molecule combined with quinazoline has drawn a colossal thought attributable to their extended applications in pharmaceutical science. Quinazolines are accounted for their enhanced natural exercises and mixes with different substitutions unite to provide information on an objective with comprehension of the molecule types that may collaborate with the objective receptors. Quinazolines are considered as a significant compound for the union of different physiological noteworthiness and pharmacologically used molecule. Quinazolines are the classes of combined heterocycles that are of extensive intrigue as a result of the various scopes of their natural properties [1]. Many substituted quinazoline subsidiaries

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have a wide scope of bioactivities, for example, antimalarials [2], anticancer [3–5], antimicrobial [6, 7], antiviral [8], antipsychotic [9], anti-obesity [10], antitubercular [11], anticonvulsant [12], antidiabetic [13], and numerous other natural exercises. Quinazoline and quinazolinone mixes are likewise utilized in planning of different useful materials for engineered science and furthermore present in different medication particles. Pyrroles and their derivatives were found most important classes of heterocyclic compounds. They prove extensive pharmacological properties such as anti-inflammatory [14], antioxidant [15], antitumor [16], antifungal [17], antibacterial [18], and immune suppressant activities [19].

This survey is an endeavor to extend the immense probability and concentrated on the different natural exercises of quinazolines and quinazolinones [20]. Quinazolinones are arranged into the accompanying 5 classes, in light of the substitution on the ring system [21]. These are 2-substituted 4(3H)-quinazolinones, 3-substituted 4(3H)-quinazolinones, 4-substitued quinazolines, 2,3-disubstituted-4(3H)-quinazolinones, and 2,4-disubstituted-4(3H)-quinazolinones. Depending on the position of the keto or oxo gathering in the quinazoline ring, these compounds might be ordered into three types [22] as pursues 2(1H)quinazolinones, 4(3H)quinazolinones, and 2,4(1H,3H)quinazolinedione quinazolinone structures; among these, 4(3H)-quinazolinones are more prevalent. Recently, few quinazoline derivatives have been approved by the FDA (Food and Drug Administration) as potential anticancer agents [23] such as lapatinib, gefitinib, afatinib, vandetanib, and erlotinib. The anticancer activity of previously reported few quinazoline-based [24, 25] and pyrrole-based [26] compounds have anticancer activity against the used cell lines and add their IC_{50} values, shown in Table 1.

The extensive literature review which reveals the effect of pyrrole derivatives in the inhibition of cancer cell proliferation has been mentioned. The quinazoline derivatives were also approved standard cytotoxic agents as previously cited. Therefore efforts have been taken to synthesised hybrid of quinazoline–pyrrole to expect a promising synergistic effect on the inhibition of cancer cell proliferation. However, there are a no literature reports on 4 substituted quinazoline–pyrrole hybrid synthesis and its anticancer activity on human breast cancer cell line and adenocarcinoma cell line. Owing to current interest on quinazoline and pyrrole as antitumor agents, our ongoing work has been continued to synthesize the quinazoline–pyrrole hybrids and to evaluate them for anticancer activity.

Methods

Reagents and conditions

The chemicals utilized for the present study, dichloromethane, pyridine, acetic anhydride, formamide, and phosphorus pentachloride, were procured from Loba Chemie PVT. LTD, Colaba, Mumbai, India. The cancer cell lines used for the study were human breast cancer cell line (MCF-7) and human lung adenocarcinoma (A-549), were sub-cultured in-house, in PGP life Sciences, Hyderabad, India. The NMR spectra were measured on a Bruker AMX 500 spectrometer (Bruker, Billerica, MA, USA) in deuterated dimethyl sulfoxide (DMSO-*d6*) and reported as δ (ppm) values relative to tetramethylsilane (TMS) at 500 and 125 MHz for ^{1}H and ^{13}C NMR,

Table 1 Anticancer activity of few previously reported quinazoline and pyrrole base derivative

Author	IUPAC name of the reported synthesized quinazoline/pyrrole derivatives	Cell line utilized	IC_{50} values (μM)	Standard IC_{50} values (μM)
Abuelizz et al. [24]	2-(Allylthio)-3-butyl-6-methylquinazolin-4(3H)-one	Cervical cancer (HeLa)	5.65	Gefitinib (4.3)
		Human breast cancer (MDA-MB231)	3.77	Gefitinib (28.33)
Abuelizz et al. [24]	Butyl-6-methyl-2-(2-methylbenzylthio)-quinazolin-4(3H)-one	Cervical cancer (HeLa)	6.3	Gefitinib (4.3)
		Human breast cancer (MDA-MB231)	4.44	Gefitinib (28.33)
Madhavi et al. [25]	(E)-1-(4-(Trifluoromethyl)phenyl)-3-(4-(quinazolin-4-ylamino)phenyl)prop-2-en-1-one	Human alveolar adenocarcinoma cell line (A459)	0.10	Combretastatin-A4 (0.11)
		Human breast adenocarcinoma cell line (MCF-7)	0.17	Combretastatin-A4 (0.18)
Madhavi et al. [25]	(E)-1-(4-Methoxyphenyl)-3-(4-(quinazolin-4-ylamino)phenyl)prop-2-en-1-one	Human alveolar adenocarcinoma cell line (A459)	2.10	Combretastatin-A4 (0.11)
		Human breast adenocarcinoma cell line (MCF-7)	0.16	Combretastatin-A4 (0.18)
Regin et al. [26]	1-(3-Aminophenyl)-1H-pyrrol-3-yl)(3,4,5-trimethoxyphenyl)-methanone	Human cervical carcinoma (HeLa)	30	Vinblastin (10)
		A-549 (human lung carcinoma)	10	Vinblastin (20.2)
Regin et al. [26]	1-(4-Methoxyphenyl)-1H-pyrrol-3-yl)(3,4,5-trimethoxyphenyl)-methanone	Human cervical carcinoma (HeLa)	150	Vinblastin (10)
		A-549 (human lung carcinoma)	80	Vinblastin (20.2)

respectively. The *J* values were recorded in Hertz. The electrospray ionization mass spectrometry (ESI-MS) spectra were recorded using a Micromass Quattro micro™ triple quadrupole tandem mass spectrometer (Waters Corp., Milford, MA, USA).

Experimental

Synthesis of 4-substituted 2-benzamido-4-(3-chloropropoxy)-5-methoxybenzoic acid analogues (3a–c)

To synthesize the compounds **3a–c**, benzoyl chloride or its 4-bromo or 4-amino derivative (**2a–c**) (0.05 mol) was added drop wise to a stirred solution of 2-amino-4-(3-chloropropoxy)-5-methoxybenzoic acid (0.05 mol) in dichloromethane/pyridine solution (70 mL) with the ratio of 90:10, and the reaction mixture was stirred at room temperature for 2 h. Water (100 mL) was then added with stirring, and the separated solid obtained by filtration was washed with water, dried, and crystallized from ethanol. [Compound 3a (M.P 266–268 °C, yield 81%), compound 3b (M.P 258–260 °C, yield 84%), compound 3b (M.P 260–261 °C, yield 77%)]

Synthesis of 7-(3-chloropropoxy)-6-methoxy-2-substituted phenyl-4H-3,1-benzoxazin-4-one derivatives (4a–c)

A mixture of corresponding 2-benzamido-4-(3-chloropropoxy)-5-methoxybenzoic acid derivative (**3a–c**) (0.03 mol) and acetic anhydride (30 mL) was heated under reflux for 1 h. Excess acetic anhydride was evaporated under reduced pressure, and the obtained solid by filtration was crystallized from ethanol. [Compound 4a (M.P 217–219 °C, yield 78%), compound 4b (M.P 222–223 °C, yield 74%), compound 4c (M.P 211–213 °C, yield 85%)]

Synthesis of 7-(3-chloropropoxy)-6-methoxy-2-phenylquinazolin-4(3H)-one derivatives (5a–c)

A mixture of corresponding 7-(3-chloropropoxy)-6-methoxy-2-phenyl-4*H*-3,1-benzoxazin-4-one derivatives (0.015 mol) and formamide (30 mL) was heated under reflux for 2 h. After cooling, the separated solid was washed with water and crystallized from acetic acid. [Compound 5a (M.P. 312–315 °C, yield 56.6%), compound 5b (M.P. 334–336 °C, yield 69.47%), compound 5c (M.P. 309–311 °C, yield 79.46%)]

Synthesis of 4-chloro-7-(3-chloropropoxy)-6-methoxy-2-substituted phenyl-quinazoline derivatives (6a–c)

The corresponding 7-(3-chloropropoxy)-6-methoxy-2-phenylquinazolin-4(3*H*)-one derivative 5 (a–c) was dissolved in *N*,*N*-dimethyl formamide (DMF) (21 mL) and was refluxed to a mixture of phosphorus oxychloride/phosphorus pentachloride ($POCl_3/PCl_5$, 2.0 mmol), with the ratio of 3:1 for 1 h of using microwave irradiation. The mixtures were then cooled and transformed into ice/water. The resulting solid products were filtered, washed with normal water, and dried. **6a–c** were crystallized from ether. [Compound 6a (M.P. 284–287 °C, yield 61.2%), compound 6b (M.P. 287–290 °C, yield 76.05%), compound 6c (M.P. 268–270 °C, yield 67.46%)]

Synthesis of 7-(3-chloropropoxy)-6-methoxy-2-phenyl-N-(3H-pyrrol-3-yl)quinazolin-4-amine derivatives (7a–i)

The corresponding 4-chloro-7-(3-chloropropoxy)-6-methoxy-2-phenylquinazoline derivatives 6(a–c) (0.01 mol) were dissolved in isopropanol (25 mL) separately. To each of the reaction, 0.1 mol of substituted pyrrole derivatives (*X* = Cl, Br, and CF_3) which was dissolved in glacial acetic acid (0.01 mol) was added and refluxed for 2 h using microwave irradiation. The reaction mixtures were cooled and were added into ice water. The solids so obtained were filtered, washed with water, dried, and recrystallized from methanol: ethyl acetate (2:2) mixture. Uncorrected melting points were determined for all the compounds (**7a–7i**) cited in Table 2 and were structurally confirmed using spectroscopic studies.

The characterization of the studied compounds (**7a–i**) was given as follows.

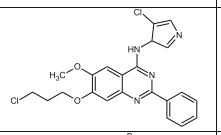
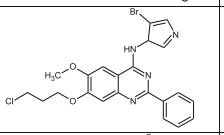
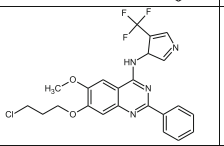
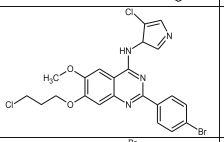
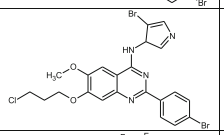
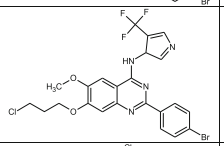
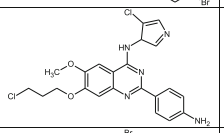
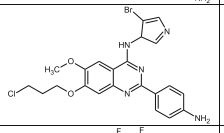
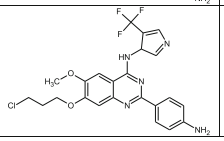
7-(3-Chloropropoxy)-N-(4-chloro-3H-pyrrol-3-yl)-6-methoxy-2-phenylquinazolin-4-amine: 7a

FT-IR (KBr) cm^{-1}: 3417 (–NH), 2968 (C–H), 1621 (C=N), 1559 (C=C), 1260 (C–O), 859 (C–Cl), 2819 (O–CH_3). ^{1}H NMR (500 MHz, DMSO-d 6): δ 8.44 (d, *J* = 9.39 Hz, 2H), 7.80 (s, 1H), 7.68 (dd, *J* = 6.51, 5.81 Hz, 1H), 7.53 (t, *J* = 7.50, 7.50 Hz, 1H), 7.53 (dd, *J* = 9.39, 7.50 Hz, 2H), 7.50 (s, 1H), 7.10 (s, 1H), 6.70 (d, *J* = 6.51 Hz, 1H), 6.70 (d, *J* = 5.81 Hz, 1H), 4.00 (t, *J* = 11.87, 5.00 Hz, 2H), 3.85 (s, 3H), 3.82 (t, *J* = 11.23, 3.76 Hz, 2H), 2.23 (tt, *J* = 13.29, 5.00, 3.76 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d 6): δ 164.41, 157.50, 154.39, 153.93, 153.88, 147.64, 136.30, 131.69, 128.63, 128.47, 124.50, 119.88, 109.79, 108.93, 107.56, 66.03, 61.23, 56.53, 42.11, 31.67; LC-MS (+ESI): m/z = 443.10 (M + H) + .

N-(4-bromo-3H-pyrrol-3-yl)-7-(3-chloropropoxy)-6-methoxy-2-phenylquinazolin-4-amine: 7b

FT-IR (KBr) cm^{-1}: 3403 (–NH), 2933 (C–H), 2813 (O–CH_3), 1612 (C=N), 1552 (C=C), 1225 (C–O), 863 (C–Cl), 640 (C–Br).; ^{1}H NMR (500 MHz, DMSO-d 6): δ 8.44 (d, *J* = 9.39 Hz, 2H), 7.80 (s, 1H), 7.68 (dd, *J* = 6.23, 6.21 Hz, 1H), 7.53 (t, *J* = 7.50, 7.50 Hz, 1H), 7.53 (dd, *J* = 9.39, 7.50 Hz, 2H), 7.50 (s, 1H), 7.10 (s, 1H), 6.70 (d, *J* = 6.23 Hz, 1H), 6.70 (d, *J* = 6.21 Hz, 1H), 4.00 (t, *J* = 11.87, 5.00 Hz, 2H), 3.85 (s, 3H), 3.82 (t, *J* = 11.23, 3.76 Hz, 2H), 2.23 (tt, *J* = 13.29, 5.00, 3.76 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d 6): δ 164.41, 154.48, 154.39, 153.93, 153.88, 147.64, 136.30, 135.79, 131.69, 128.63, 128.47, 114.08, 109.79, 108.93, 107.56, 66.03, 56.53, 54.58, 42.11, 31.67; LC-MS (+ESI): m/z = 487.05 (M + H) +.

Table 2 Synthesized quinazoline–pyrrole hybrid compounds

S.No	Structure	IUPAC	M.F	M.W	M.P in °C	Yield in %
7a		7-(3-chloropropoxy)-*N*-(4-chloro-3*H*-pyrrol-3-yl)-6-methoxy-2-phenylquinazolin-4-amine.	$C_{22}H_{20}Cl_2N_4O_2$	443.32	285.3-287.6	63.7
7b		*N*-(4-bromo-3*H*-pyrrol-3-yl)-7-(3-chloropropoxy)-6-methoxy-2-phenylquinazolin-4-amine	$C_{22}H_{20}BrClN_4O_2$	487.77	293.7-294.5	59.4
7c		*N*-(4-trifluoromethyl-3*H*-pyrrol-3-yl)-7-(3-chloropropoxy)-6-methoxy-2-phenylquinazolin-4-amine	$C_{23}H_{20}ClF_3N_4O_2$	476.87	276.4-278.3	51.2
7d		2-(4-bromophenyl)-7-(3-chloropropoxy)-*N*-(4-chloro-3*H*-pyrrol-3-yl)-6-methoxyquinazolin-4-amine	$C_{22}H_{19}BrCl_2N_4O_2$	522.22	302.2-304.7	62.3
7e		*N*-(4-bromo-3*H*-pyrrol-3-yl)-2-(4-bromophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine	$C_{22}H_{19}Br_2ClN_4O_2$	566.67	293.6-294.8	67.5
7f		*N*-(4-trifluoromethyl-3*H*-pyrrol-3-yl)-2-(4-bromophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine	$C_{23}H_{19}BrClF_3N_4O_2$	555.77	296.1-297.9	57.4
7g		2-(4-aminophenyl)-7-(3-chloropropoxy)-*N*-(4-chloro-3*H*-pyrrol-3-yl)-6-methoxyquinazolin-4-amine	$C_{22}H_{21}Cl_2N_5O_2$	458.34	289.3-291.4	68.8
7h		*N*-(4-bromo-3*H*-pyrrol-3-yl)-2-(4-aminophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine	$C_{22}H_{21}BrClN_5O_2$	502.79	296.5-297.4	69.5
7i		*N*-(4-trifluoromethyl-3*H*-pyrrol-3-yl)-2-(4-aminophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine	$C_{23}H_{21}ClF_3N_5O_2$	491.89	309.6-311.4	62.6

N-(4-trifluoromethyl-3H-pyrrol-3-yl)-7-(3-chloropropoxy)-6-methoxy-2-phenylquinazolin-4-amine: 7c

FT-IR (KBr) cm^{-1}: 3518 (–NH), 2863 (C–C), 2895 (O–CH_3), 1627 (C=N), 1559 (C=C), 1208 (C–O), 843 (C–Cl), 640 (C–Br), 1250 (C–F) ^{1}H NMR (500 MHz, DMSO-d 6): δ 8.44 (d, *J* = 9.39 Hz, 2H), 7.80 (s, 1H), 7.68 (dd, *J* = 5.92, 5.27 Hz, 1H), 7.53 (t, *J* = 7.50, 7.50 Hz, 1H), 7.53 (dd, *J* = 9.39, 7.50 Hz, 2H), 7.50 (s, 1H), 7.10 (s, 1H), 6.70 (d, *J* = 5.92 Hz, 1H), 6.50 (d, *J* = 5.27 Hz, 1H), 4.00 (t, *J* = 11.87, 5.00 Hz, 2H), 3.85 (s, 3H), 3.82 (t, *J* = 11.23, 3.76 Hz, 2H), 2.23 (tt, *J* = 13.29, 5.00, 3.76 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d 6): δ 164.41, 164.07, 164.04, 164.01, 163.97, 154.39, 153.93, 153.88,147.64, 143.15, 143.12, 143.08, 143.05, 136.30, 131.69, 130.78, 129.90, 129.64, 129.39, 129.13, 128.63, 128.47, 126.50, 124.35, 109.79, 108.93, 107.56, 70.92, 70.91, 70.89, 70.88, 66.03, 56.53, 42.11, 31.67; LC-MS (+ESI): m/z = 477.12 (M + H) +.

2-(4-Bromophenyl)-7-(3-chloropropoxy)-N-(4-chloro-3H-pyrrol-3-yl)-6-methoxyquinazolin-4-amine: 7d

FT-IR (KBr) cm^{-1}: 3416 (–NH), 2885 (C–C), 2812 (O–CH_3), 1621 (C=N), 1553 (C=C), 1206 (C–O), 840 (C–Cl), 632 (C–Br); ^{1}H NMR (500 MHz, DMSO-d 6): δ 8.10 (d, *J* = 9.21 Hz, 2H), 7.80 (s, 1H), 7.75 (d, *J* = 9.21, 2H), 7.68 (dd, *J* = 6.51, 5.81 Hz, 1H), 7.50 (s, 1H), 7.10 (s, 1H), 6.70 (d, *J* = 6.51 Hz, 1H), 6.70 (d, *J* = 5.81 Hz, 1H), 4.00 (t, *J* = 11.87, 5.00 Hz, 2H), 3.85 (s, 3H), 3.82 (t, *J* = 11.23, 3.76 Hz,

2H), 2.23 (tt, *J* = 13.29, 5.00, 3.76 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d 6): δ 164.41, 157.50, 154.39, 153.93, 153.88, 147.64, 135.83, 131.61, 130.13, 125.30, 124.50, 119.88, 109.79,108.93, 107.56, 66.03, 61.23, 56.53, 42.11, 31.67; LC-MS (+ESI): m/z = 521.01 (M + H) +.

N-(4-bromo-3H-pyrrol-3-yl)-2-(4-bromophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine: 7e

FT-IR (KBr) cm^{-1}: 3407 (–NH), 2960 (C–H), 2807 (O–CH_3), 1625 (C=N), 1532 (C=C), 1216 (C–O), 861 (C–Cl), 1220 (C–F).; ^{1}H NMR (500 MHz, DMSO-d 6): δ 8.10 (d, *J* = 9.21 Hz, 2H), 7.80 (s, 1H), 7.75 (d, *J* = 9.21, 2H), 7.68 (dd, *J* = 6.23, 5.21 Hz, 1H), 7.50 (s, 1H), 7.10 (s, 1H), 6.70 (d, *J* = 6.23 Hz, 1H), 6.70 (d, *J* = 6.21 Hz, 1H), 4.00 (t, *J* = 11.87, 5.00 Hz, 2H), 3.85 (s, 3H), 3.82 (t, *J* = 11.23, 3.76 Hz, 2H), 2.23 (tt, *J* = 13.29, 5.00, 3.76 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d 6): δ 164.41, 154.48, 154.39, 153.93, 153.88, 147.64, 135.83, 135.79, 131.61, 130.13, 125.30, 114.08, 109.79,108.93, 107.56, 66.03, 56.53, 54.58, 42.11, 31.67; LC-MS (+ESI): m/z = 564.96 (M + H) +.

N-(4-trifluoromethyl-3H-pyrrol-3-yl)-2-(4-bromophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine: 7f

FT-IR (KBr) cm^{-1}: 3511 (–NH), 2863 (C–C), 2810 (O–CH_3), 1625 (C=N), 1533 (C=C), 1218 (C–O), 843 (C–Cl), 633 (C–Br), 1252 (C–F). ^{1}H NMR (500 MHz, DMSO-d 6): δ 8.10 (d, *J* = 9.21 Hz, 2H), 7.80 (s, 1H), 7.75 (d, *J* = 9.21, 2H), 7.68 (dd, *J* = 5.92, 5.27 Hz, 1H), 7.50 (s, 1H), 7.10 (s, 1H), 6.70 (d, *J* = 5.92 Hz, 1H), 6.50 (d, *J* = 5.27 Hz, 1H), 4.00 (t, *J* = 11.87, 5.00 Hz, 2H), 3.85 (s, 3H), 3.82 (t, *J* = 11.23, 3.76 Hz, 2H), 2.23 (tt, *J* = 13.29, 5.00, 3.76 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d 6): δ 164.41, 164.07, 164.04, 164.01, 163.97, 154.39, 153.93, 153.88, 147.64, 143.15, 143.12, 143.08, 143.05, 135.83, 131.61, 130.78, 130.13, 129.90, 129.64, 129.39, 129.13, 128.64, 126.49, 125.30, 124.35, 109.79, 108.93, 107.56, 70.92, 70.91, 70.89, 70.88, 66.03, 56.53, 42.11, 31.67; LCMS (+ESI): m/z = 555.04 (M + H) +.

2-(4-Aminophenyl)-7-(3-chloropropoxy)-N-(4-chloro-3H-pyrrol-3-yl)-6-methoxyquinazolin-4-amine:7g

FTIR (KBr) cm^{-1}: 3508 (–NH), 2876 (C–C), 2810 (O–CH_3), 1625 (C=N), 1529 (C=C), 1228 (C–O), 830 (C–Cl), ^{1}H NMR (500 MHz, DMSO-d 6): δ 7.85 (d, *J* = 8.99 Hz, 2H), 7.80 (s, 1H), 7.68 (dd, *J* = 6.51, 5.81 Hz, 1H), 7.50 (s, 1H), 7.10 (s, 1H), 6.70 (d, *J* = 6.51 Hz, 1H), 6.70 (d, *J* = 5.81 Hz, 1H), 6.60 (d, *J* = 8.99 Hz, 2H), 4.05 (s, 2H), 4.00 (t, *J* = 11.87, 5.00 Hz, 2H), 3.85 (s, 3H), 3.82 (t, *J* = 11.23, 3.76 Hz, 2H), 2.23 (tt, *J* = 13.29, 5.00, 3.76 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d 6): δ 164.41, 157.50, 154.39, 153.93, 153.88, 150.54, 147.64, 130.54, 129.38, 124.50, 119.88, 114.94, 109.79, 108.93, 107.56, 66.03, 61.23, 56.53, 42.11, 31.67; LC-MS (+ESI): m/z = 458.11 (M + H) +.

N-(4-bromo-3H-pyrrol-3-yl)-2-(4-aminophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine: 7h

FTIR (KBr) cm^{-1}: 3533 (–NH), 2875 (C–C), 2821 (O–CH_3), 1621 (C=N), 1531 (C=C), 1211 (C–O), 843 (C–Cl), 639 (C–Br).^{1}H NMR (500 MHz, DMSO-d 6): δ 7.85 (d, *J* = 8.99 Hz, 2H), 7.80 (s, 1H), 7.68 (dd, *J* = 6.23, 6.21 Hz, 1H), 7.50 (s, 1H), 7.10 (s, 1H), 6.70 (d, *J* = 6.23 Hz, 1H), 6.70 (d, *J* = 6.21 Hz, 1H), 6.60 (d, *J* = 8.99 Hz, 2H), 4.05 (s, 2H), 4.00 (t, *J* = 11.87, 5.00 Hz, 2H), 3.85 (s, 3H), 3.82 (t, *J* = 11.23, 3.76 Hz, 2H), 2.23 (tt, *J* = 13.29, 5.00, 3.76 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d 6): δ 164.41, 154.48, 154.39, 153.93, 153.88, 150.54, 147.64, 135.79, 130.54, 129.38, 114.94, 114.08, 109.79, 108.93, 107.56, 66.03, 56.53, 54.58, 42.11, 31.67; LC-MS (+ESI): m/z = 502.06 (M + H) +.

N-(4-trifluoromethyl-3H-pyrrol-3-yl)-2-(4-aminophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine: 7i

FTIR (KBr) cm¯1: 3439 (–NH), 2876 (C–C), 2819 (O–CH_3), 1625 (C=N), 1533 (C=C), 1228 (C–O), 848 (C–Cl), 631 (C–Br), 1255 (C–F). ^{1}H NMR (500 MHz, DMSO-d 6): δ 7.85 (d, *J* = 8.99 Hz, 2H), 7.80 (s, 1H), 7.68 (dd, *J* = 5.92, 5.27 Hz, 1H), 7.50 (s, 1H), 7.10 (s, 1H), 6.70 (d, *J* = 5.92 Hz, 1H), 6.60 (d, *J* = 8.99 Hz, 2H), 6.50 (d, *J* = 5.27 Hz, 1H), 4.05 (s, 2H), 4.00 (t, *J* = 11.87, 5.00 Hz, 2H), 3.85 (s, 3H), 3.82 (t, *J* = 11.23, 3.76 Hz, 2H), 2.23 (tt, *J* = 13.29, 5.00, 3.76 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d 6): δ 164.41, 164.07, 164.04, 164.01, 163.97, 154.39, 153.93, 153.88, 150.54, 147.64, 143.15, 143.12, 143.08, 143.05, 130.78, 130.54, 129.90, 129.64, 129.39, 129.13, 128.64, 126.50, 124.35, 114.94, 109.79, 108.93, 107.56, 70.92, 70.91, 70.89, 70.88, 66.03, 56.53, 42.11, 31.67; LC-MS (+ESI): m/z = 492.14 (M + H) +.

Antitumor activity

Cell culture and sub-culture

To carry out the cell culture [24], the human breast cancer cell line (MCF-7) and human lung adenocarcinoma (A-549) were developed in 75-cm^2 bottle inclined necked vented carafes (Corning) with DMEM, and the cells were kept up in a humidified environment of 5% CO_2 at 37 °C. Cells (sections 30–50) were developed in Dulbecco's Modified Eagles medium (Gibco Invitrogen, Paisley, UK) enhanced with 10% fetal cow-like serum, 1% insignificant amino acids, 1% penicillin (1000 U/mL), 1% streptomycin (1000 μg/mL), and 1% amphotericin (250 U/mL). The phones were passage enzymatically with 0.25% trypsin-1 mM EDTA and sub-refined on 75cm^2 plastic cups at a thickness of 2.2 × 104 cells/cm^2. Culture medium was supplanted like clockwork. Cell conversion (80%) was affirmed by minute recognition.

Trials were performed 24 h present seeding on counteract cell separation. All the molecules used were of 95–97% pure and were gaged by HPLC and verified by mass spectrometry.

For subculture, 75-cm^2 flask was used. Cell layer was briefly rinsed with 0.25% (w/v) trypsin-0.53 mM EDTA answer to expel all hints of serum that contains trypsin inhibitor. Accurately 2.0 to 3.0 mL of trypsin-EDTA arrangement was added to cup and watched cells under a rearranged magnifying instrument until cell layer is scattered (for the most part inside 5 to 15 min). Six to 8.0 mL of complete development medium was included and suctioned the cells by tenderly pipetting. Proper aliquots of the cell suspension were added to new culture vessels. Societies can be built up between 2 × 103 and 1 × 104 viable cells/cm^2. Try not to surpass 7 × 104 cells/cm^2. Societies were brooded at 37 °C. Cultures are maintained at a cell concentration between 6×10^3 and 6×10^4 cell/cm^2. A sub cultivation ratio of 1:3 to 1:8 is maintained. Medium renewal was performed at 2 to 3 times per week.

Screening of novel 2-substituted-4-anilinoquinazolines against morphology of MCF-7 and A-549

Morphological observations of MCF-7 and A-549 cells treated with different compounds for cytotoxicity were done to determine the changes induced by the standard and the test compounds. MCF-7 and A-549 cell were treated with 100 μM concentration of novel 2-substituted 4-anilinoquinazolines (**7a–7i**) which were characterized using NMR, mass, and IR spectral techniques prior to use for morphological study. As a part of anti-cancer evaluation, we have screened all 9 newly synthesized molecules of quinazolines against morphological behavior of MCF-7 and A-549. Cells were observed for 24, 48, and 72 h, after treatment of all test molecules. Images were taken by Axiovert 200M phase contrast microscope at the magnification of ×10. Axiovision Rel.4.2 programming was utilized to get the pictures.

Determination of cytotoxic concentration of 7d, 7f, 7 g, and 7 h, against MCF-7 and A-549 using MTT Assay

Plate A-549 and MCF-7 cells (100 μL per well) in a 96-well tissue culture plate. (The quantity of cells can differ from 1000 to 80,000 for each well. The volume can differ from 50 to 150 μL, albeit 100 μL is utilized in this trial.) The test molecules (**7d**, **7f**, **7 g**, and **7 h**) were included and controlled and brooded the cells for the 24 h timeframe. A volume of 20 μL in phosphate-buffered saline (PBS) or culture medium is suggested for the test mixes and controls. The Control Reagent can be helpfully reconstituted with 5 mL PBS. Fifteen microliter (per 100 μL cell culture) of reagent per well was included and brooded for 4 h at 37 °C. The volume of the reagent ought to be balanced relying upon the volume of cell culture. One hundred microliter of the solubilizer was included to each well and blended delicately on an orbital shaker for 1 h at room temperature. The volume of the solubilizer ought to be balanced relying upon the volume of cell culture. (On the off chance that precipitation happens in the solubilizer, place the jug in a warm water shower or at 37 °C and shake to disintegrate accelerates.) The absorbance was measured at OD 570 nm for each well on an absorbance plate reader. The study [27] was performed in triplicate. Percent proliferation inhibition was calculated using the formula

$$\text{Viability cell inhibition } (\%) = 100 - [\,(\text{At} - \text{Ab})/(\text{Ac} - \text{Ab})] \times 100$$

At = Absorbance of the test compound, Ab = Absorbance of the blank, Ac = Absorption of control.

IC_{50} values were calculated by analyzing the relationship between concentrations and percent (%) inhibitions using the GraphPad Prism 7 version 7.00 for Windows, GraphPad Software, La Jolla, CA.

Results

In the present study, a series of 2 substituted 4 anilinoquinazolines **(7a–i)** has been synthesized from the corresponding 4 chloro 7-(3 chloropropoxy) 6-methyl-2 phenyl quinazoline 4 (3H) amine derivatives **(6a–c)** condensing with substituted pyrrole derivatives. The newly synthesized nine quinazoline-pyrrole hybrids have been characterized and confirmed using FT-IR (Fourier transform infrared spectroscopy) for the functional groups, ^{1}H NMR (nuclear magnetic resonance) for the equivalent protons, ^{13}C NMR for the presence of number of equivalent ^{13}C atoms, and mass spectroscopy for the determination of molecular weight. The details of the reaction scheme are shown in Fig. 1. The melting points and percentage of yield are summarized in Table 2. The obtained percentage of yield was satisfactory. The characterization of the synthesized compounds has been explained and shown in the "Experimental" section.

Result of biological evaluation

The result of the screening of novel 2-substituted 4-anilinoquinazolines against morphology of MCF-7 and A-549 shows that few synthesized molecules have shown significant cytotoxic effect on both MCF-7 and A-549 cells in a time-dependent manner.

The compounds **7d**, **7f**, **7 g**, and **7 h** moderately inhibit the proliferation of A-549 cells and therefore exhibited moderate activity against A-549 cells. The compounds **7d** and **7f** were also found effective in MCF-7 cells as the morphological figures show that there is a prominent inhibition of the MCF-7 breast cancer cell proliferation.

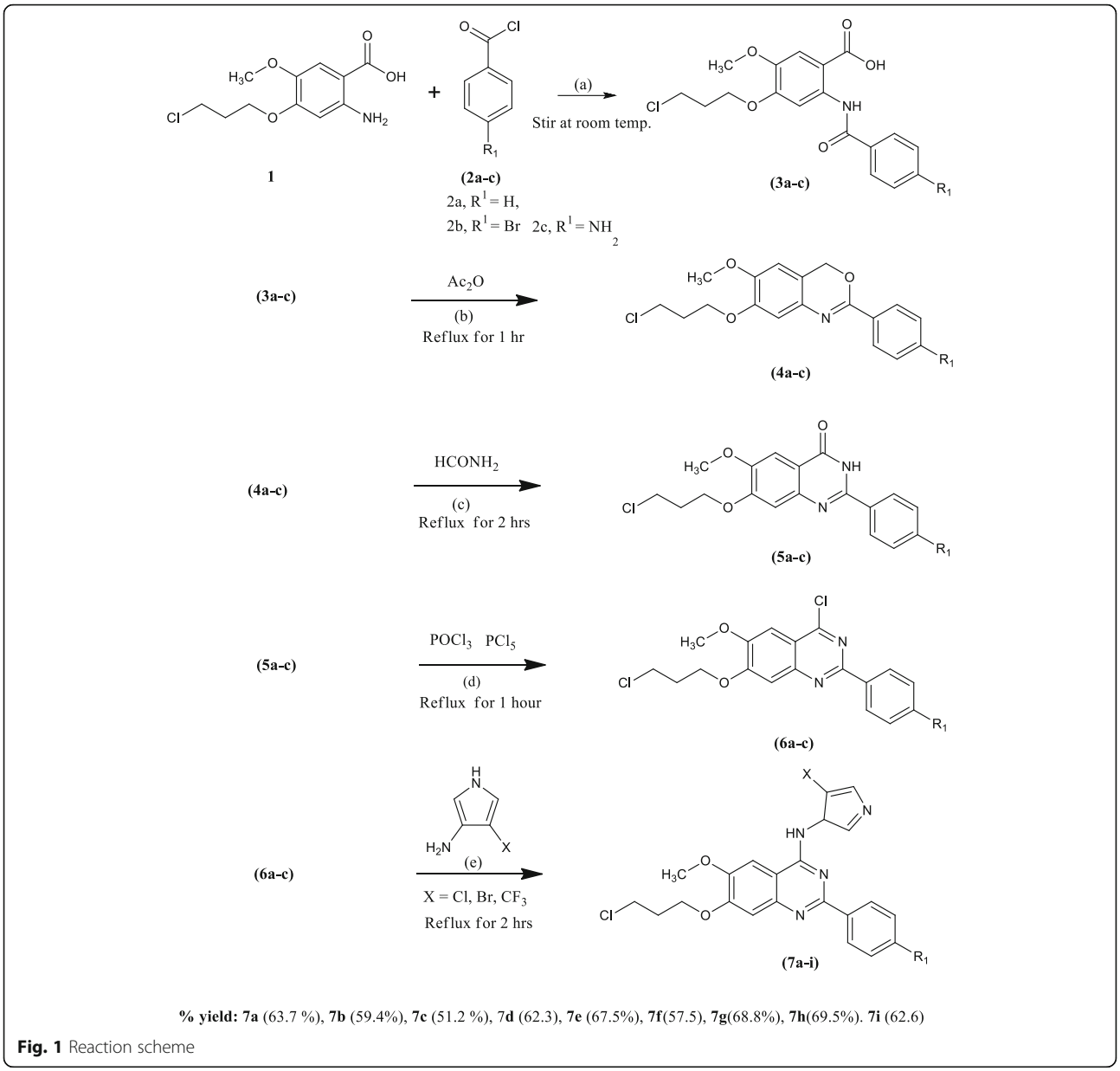


Fig. 1 Reaction scheme

Other molecules have not shown any inhibition of cell proliferation at any time point of observation as shown in Fig. 2. So further anticancer studies that were carried out using the molecules show better results in morphological study.

MTT assay was conducted for the compounds **7d**, **7f**, **7 g**, and **7 h**; IC_{50} values were calculated for the tested compound and also standard doxorubicin. In A-549 adenocarcinoma cell line, the calculated IC_{50} values (μM) for the compounds **7d**, **7f**, **7 g**, and **7 h** were 49.93 ± 2.23, 49.94 ± 4.56, 54.65 ± 3.06, and 60.62 ± 2.58 at 24 h study, respectively. The standard doxorubicin shows 52.37 ± 3.69 as an IC_{50} value for 24 h. This study was further extended to 48 and 72 h. In MCF-7 breast cancer cell line, only two compounds were tested based on the report of morphological study. The calculated IC_{50} values for the compounds **7d** and **7f** were 43.99 ± 1.65 and 47.70 ± 3.68 for 24 h study, respectively. In doxorubicin, it shows 54.29 ± 2.46 at 24 h. The IC_{50} values at 48 and 72 h have been included in Table 3. Results of cytotoxic activity are also depicted in Figs. 3 and 4.

Discussion

Quinazoline derivatives previously have been demonstrated as potent anticancer agents [28]. A series of 2 substituted 4 anilinoquinazolines (**7a**–**i**) has been synthesized from the corresponding 4 chloro 7-(3 chloropropoxy) 6-methyl-2 phenyl quinazoline 4 (3H) amine

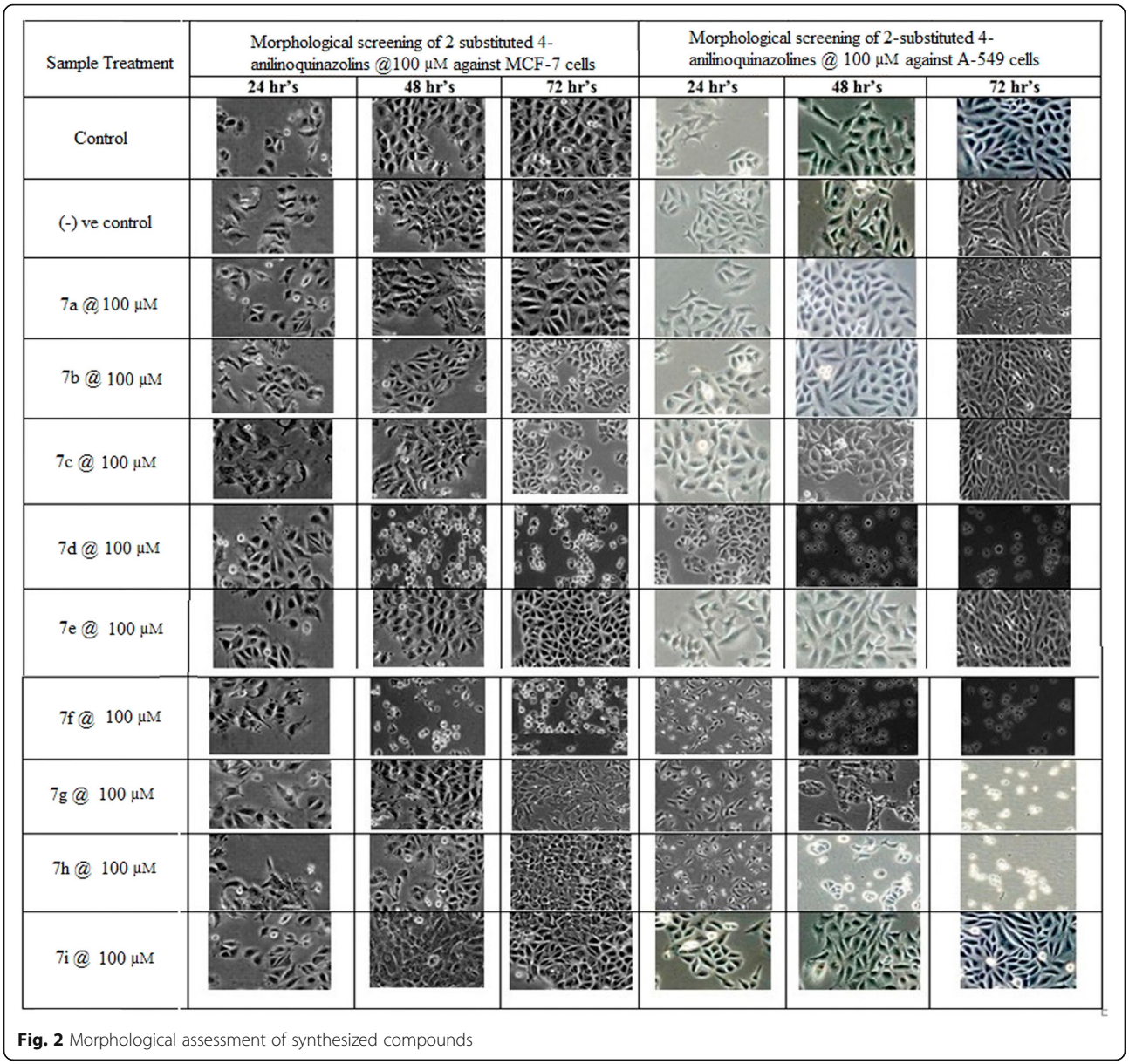


Fig. 2 Morphological assessment of synthesized compounds

Table 3 Anticancer activity of targeted quinazoline derivatives

Compound code	24 h	48 h	72 h
IC 50 (μM) of A-549 human adenocarcinoma cells			
7d	49.93 ± 2.23	43.99 ± 1.56	41.05 ± 0.59
7f	49.94 ± 4.56	49.28 ± 2.54	45.54 ± 1.26
7 g	54.65 ± 3.06	50.55 ± 3.54	46.93 ± 1.69
7 h	60.62 ± 2.58	59.09 ± 1.26	48.62 ± 0.93
Doxorubicin	52.37 ± 3.69	49.13 ± 2.9	48.62 ± 2.56
IC 50 (μM) of MCF-7 human breast cancer cells			
7d	43.99 ± 1.65	41.56 ± 1.43	40.64 ± 0.89
7f	47.70 ± 3.68	46.37 ± 1.88	44.98 ± 1.96
Doxorubicin	54.29 ± 2.46	50.67 ± 1.03	47.62 ± 2.56

derivatives (**6a**–**c**). The results of all the spectral analysis were found suitable and justified the structures of the synthesized compounds under study. Morphological studies clearly shows about the moderate inhibition of A-549 cell proliferation due to the effect of **7d**, **7f**, **7g**, and **7h** compounds and only two compounds **7d** and **7f** were able to inhibit MCF-7 cell proliferation.

After morphological study, the results of the cytotoxic activity of the tested compounds using MTT assay confirmed that the compounds **7d**, **7f**, **7g**, and **7h** exhibited satisfactory cytotoxic activity in A-549 lung adenocarcinoma cancer cells. It was indicated that the presence of para substituted phenyl group at 2nd position of the quinazoline ring is essential for the anticancer activity because the newly synthesized compounds with only

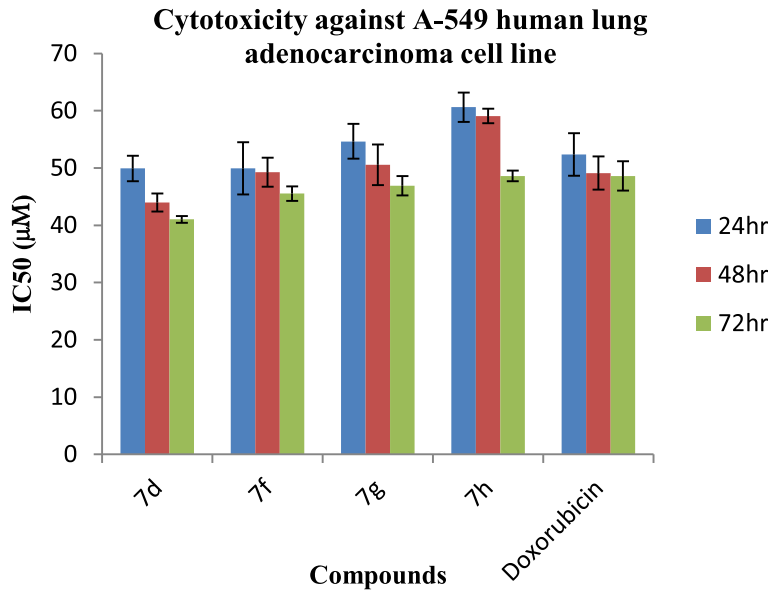


Fig. 3 Cytotoxic potential of new quinazolines in adenocarcinoma cell lines

phenyl substitution at 2nd position of the quinazoline ring were found inactive against both A-549 adenocarcinoma cancer cells and MCF-7 human breast cancer cell lines. Only two compounds **7d** and **7f** were found active against MCF-7 human breast cancer cell lines. The presence of significant electronegative group, trifluoromethyl ($-CF_3$) at the ortho position of the substituted pyrrole ring in the compound **7f**, was found to be a major determinant of the cytotoxic activity, specifically in the MCF-7 human breast cancer cell lines. In the compound **7d**, the presence of electronegative as well as inductively electron withdrawing chlorine group

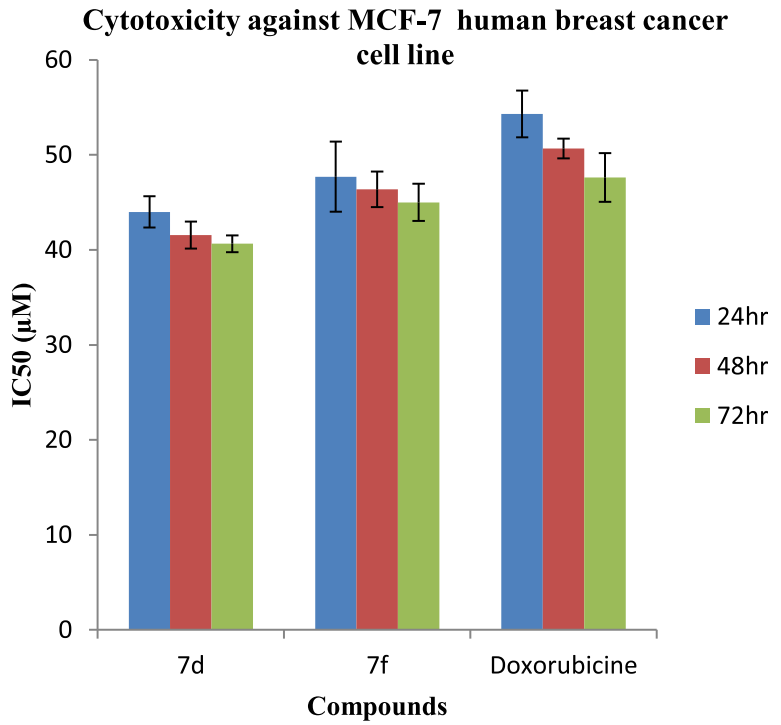


Fig. 4 Cytotoxic potential of new quinazolines in human breast cancer cell lines

(–Cl), at the ortho position of the pyrrole ring which is substituted in the 4th position of the quinazoline ring, and the presence of electron-donating amino group ($–NH_2$) reduced the hydrophobicity [29] of the 2 substituted phenyl ring in the quinazoline moiety. Therefore, the compound **7d** was found as a determinant for the cytotoxic potential on MCF-7 human breast cancer cell lines. Out of nine synthesized compounds, only four compounds (**7d**, **7f**, **7g**, and **7h**) were found to have better cytotoxic potential in comparison to standard doxorubicin.

Conclusion

The empirical evidences of the present study revealed that the newly synthesized quinazoline-pyrrole hybrid compounds **7d**, **7f**, **7g**, and **7h** showed good activity against the A-459 human adenocarcinoma cell lines. Among the compounds, 7d which had lowest IC_{50} value in relation to doxorubicin was identified as most potent. In MCF-7 breast cancer cell lines, the compounds **7d** and **7f** showed good activity. The obtained significant and remarkable cytotoxic effects using substituted pyrrole and 2-substituted quinazolines could be consider as a useful template for further derivatization and designed of modification to achieve more compounds with potent cytotoxic effects.

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Authors' contributions

"RB" and "SS" designed the entire project work after extensive literature review. "PM" and "RB" carried out the synthesis of the compounds. "RB" and "SS" elucidate the structures using FTIR, ^{13}C-NMR, and mass spectra. RR, PM, and RB carried out the cytotoxic evaluation. "PM" has drafted the manuscript and subsequently revised it. The research work was performed in collaboration between all the authors. All the authors have read and approved the final manuscript.

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Availability of data and materials

All data and material is available upon request

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interest.

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