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ARTICLE

Design, synthesis, anticancer, antibacterial, and antifungal evaluation of 4-aminoquinoline-1,3,5-triazine derivatives

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Abstract

A series of 4-aminoquinoline 1,3,5-triazine derivatives were synthesized and evaluated for anticancer activity against cancer cell lines HeLa, MCF-7, HL-60, HepG2 where these derivatives exert significant anticancer activity. The molecules found nontoxic against MCF-12A. The molecules also showed potent inhibition of EGFR-TK as compared to eroltinib in enzyme-based assay. The newly synthesized derivatives were screened for their *in vitro* antibacterial and antifungal activity against *Bacillus subtilis, Bacillus cereus*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus* using cefixime and fluconazole as standard. Antibacterial screening results suggest that compound **7c** showed potent activity against *S. aureus, P. aeruginosa*, and *P. vulgaris*. In antifungal screening, compound **7b** showed significant activity against *A. niger*, *A. fumigatus* and moderate activity against *C. albicans*.

1 | INTRODUCTION

Cancer has a very devastating effect on human health because of high morbidity and mortality. It was responsible for 9.6 million deaths across the world, the second most reason of death after cardiovascular diseases.[1,2] Despite recent advances to treat cancer with personalized therapy and novel immunotherapeutics, the overall survival rate is not significantly improved.[3,4] The generation of resistance against the drugs to treat cancer is the major hurdle for the failure of the current therapeutic regimes.[5–7] Various studies have shown that the impact of cancer is truly shocking in low- or middle-income countries because of fewer resources are available for diagnostics and therapeutic purpose.[8] The role of kinases has been widely implicated in propagation, survival, motility, metabolism, angiogenesis, and evasion of antitumor immune responses.[9] Thus, inhibition of kinases is considered as a novel strategy to curb the menace of cancer, as a result, more than 25 anticancer drugs that target kinases have been approved and numerous agents are in various stages of clinical trial.[10] The epidermal growth factor receptors (EGFRs) are a large family of receptor tyrosine kinases (TK) over-expressed in several types of cancer, including breast, lung, esophageal, and head and neck.[11] The EGFR undergoes conformational changes after binding with the ligands (e.g., EGF) causing intermolecular autophosphorylation, which plays important role for cellular survival, and proliferation via mitogen-activated protein kinase pathway.[12,13] The cancer patients are more prone to microbial infection due to immuno-compromised host defenses system, disruption in the barriers to infection, and shifts in the microbial flora.[14] Thus, novel agents are urgently needed, which can simultaneously able to control the cancer progression as well as antimicrobial growth.

Heterocyclic molecules are well known for their importance in medicinal chemistry because of diverse pharmacological benefits and ease of derivatization for the generation of newer scaffolds.[15] In this regard, 4-aminoquinoline and

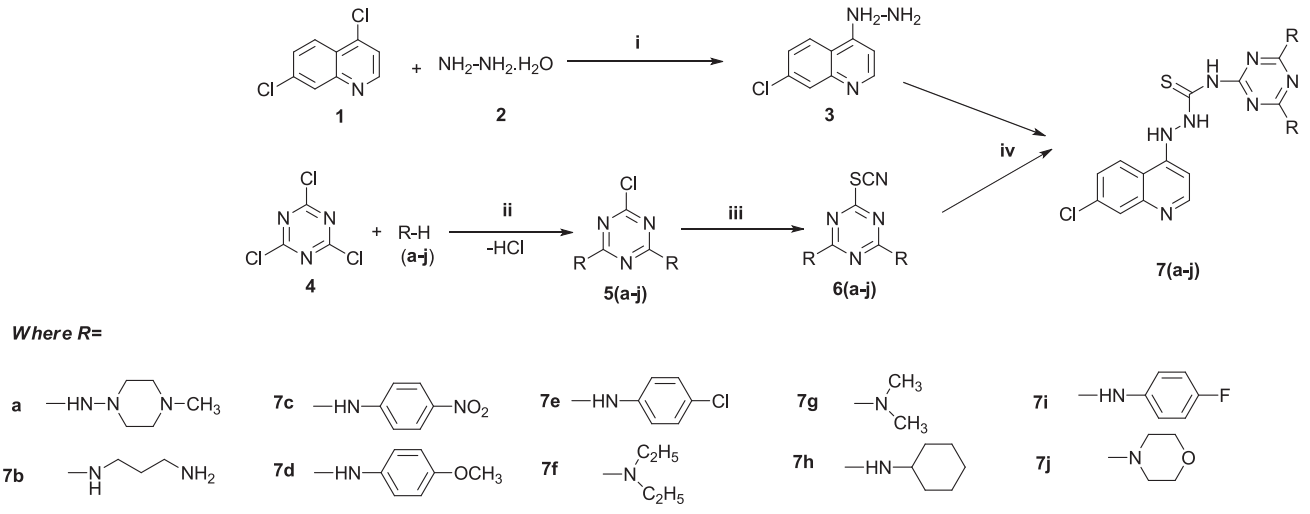
1,3,5-triazine have gained a lot of attention from the researchers because of an extensive array of pharmacological properties, such as antibacterial,[16,17] antimalarial,[18,19] anti-inflammatory,[20] antifungal,[21] anti-HIV,[22] anti-diabetic,[23] and against cystic fibrosis.[24] More recently, these pharmacophores showed excellent anticancer activity.[25] Thus, in the present study, we intended to synthesize a single skeleton comprised of 4-aminoquinoline and 1,3,5-triazine with subsequent screening against cancer, bacterial and fungal micro-organisms.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthesis of novel hybrid 4-aminoquinoline 1,3,5-triazine derivatives **7a-j** was accomplished in four steps. Initially, the synthesis of 7-chloro-4-hydrazinylquinoline (**3**) was achieved by reacting 4,7-dichloroquinoline with (**1**) hydrazine hydrate (**2**) in the presence of ethanol. The second step corresponds to the synthesis of di-substituted 1,3,5-triazines **5**(**a-j**) by nucleophilic substitution of the Cl atom of the 2,4,6-trichloro-1,3,5-triazine (**4**) with various aliphatic and aromatic amines (**a-j**), in presence of $NaHCO_3$ as a base. Whereas, synthesis of tri-substituted 1,3,5-triazine derivatives **6(a-j)** by the nucleophilic substitution of Cl atom of di-substituted 1,3,5-triazine derivatives **5(a-j)** with potassium thiocyanate in presence of a few pieces of tin granules as catalyst. The last step corresponds to the synthesis of title compounds **7**(**a-j**) upon reacting compound **3** with **6**(**a-j**) in the presence of dry acetone, as shown in Scheme 1.

The title hybrid derivatives were synthesized in 65%–82% yield. Furthermore, the structure of title compounds and intermediate were established by different spectroscopic analysis. The FTIR spectra of title hybrid compounds **7**(**a-j**) were found in 3408–3392 cm^{-1}, which can be attributed to aromatic N—$H_{stretch}$. The presence of C—N group appeared at 1637–1623 cm^{-1}. The peak such as 1169–1165 was attributed to C═S group. ^1H NMR spectra of the title compounds **7**(**a-j**) showed proton of quinoline moiety as doublets with chemical shift ranging from 8.63 to 6.76 ppm. The aliphatic proton of the morpholine appeared as a multiplet in the range at 3.74–3.63 ppm. The methylene proton of cyclohexene moiety appeared as a multiplet at 1.71–1.11 ppm. Furthermore, the aromatic proton of *para*-nitroaniline substituted 1,3,5-triazine derivative (**7c**) appeared as doublets at 8.01–6.78 ppm. The methoxy of the compound **7d** appeared as a singlet at 3.74 ppm. The aliphatic protons of the methyl group of the ethylene moiety were appeared as triplets at 1.23 ppm, while a methylene group was observed at 3.26 ppm as quartet. The N—H proton appeared at 3.96–3.98 ppm as singlet. The methyl proton appeared as a singlet at the chemical shift range of 3.06 ppm (**7g**). The ^{13}C NMR spectrum of title compounds **7**(**a-j**) showed the presence of nine carbon of the quinoline ring in the region of 152.7–113.1 ppm. The piperazine methylene carbons were observed at 59.5 and 55.1 ppm. The two carbon of the tri-substituted 1,3,5-triazine ring was observed at 176.4 and 171.1 ppm. Finally, the structure of the title compounds was confirmed by Mass and elemental analysis.



SCHEME 1 Reagents and conditions: R-H (a-j) various amines (i) ethanol, reflux for 9 hours (ii) acetone, stir 6 hours at 40–45°C, $NaHCO_3$ (iii) KSCN, 1,4 dioxane, reflux at 105°C for 7–8 hours, K_2CO_3, tin granules (iv) dry acetone, reflux for 10–11 hours at 60–65°C

TABLE 1 Anticancer activity of target compounds **7** (**a**-**j**)

	IC_{50} (in μM)				
Compounds	**HeLa**	**MCF-7**	**HL-60**	**HepG2**	**MCF-12A**
7a	51.2 ± 0.33	61.4 ± 0.34	47.6 ± 0.42	63.3 ± 0.63	Nontoxic
7b	81.8 ± 0.34	76.5 ± 0.54	NA	NA	Nontoxic
7c	44.5 ± 0.43	52.2 ± 0.33	40.3 ± 0.34	56.4 ± 0.30	Nontoxic
7d	58.2 ± 0.27	64.5 ± 0.32	53.3 ± 0.22	68.3 ± 0.48	Nontoxic
7e	32.4 ± 0.22	32.3 ± 0.43	26.3 ± 0.23	45.3 ± 0.33	Nontoxic
7f	73.3 ± 0.45	79.5 ± 0.40	63.0 ± 0.34	74.2 ± 0.46	Nontoxic
7g	78.2 ± 0.34	NA	69.3 ± 0.43	NA	Nontoxic
7h	69.4 ± 0.34	72.2 ± 0.32	59.2 ± 0.45	71.2 ± 0.41	Nontoxic
7i	41.2 ± 0.24	45.2 ± 0.22	34.3 ± 0.34	51.2 ± 0.35	Nontoxic
7j	54.4 ± 0.34	61.0 ± 0.32	48.3 ± 0.31	63.4 ± 0.33	Nontoxic
Cisplatin	31.3 ± 0.55	22.5 ± 0.86	14.3 ± 0.55	26.4 ± 0.38	Nontoxic

NA: Not active.

TABLE 2 EGFR tyrosine kinase inhibitory activity of selected derivatives at 10 μM

Compounds	**Percent of inhibition**
7a	45.1
7c	96.3
7e	90.5
7i	71.6
7j	65.2
Erlotinib	100

2.2 | Anticancer activity

The MTT assay was used to evaluate the *in vitro* cytotoxic activity synthesized derivatives against various cell lines such as HeLa (cervical cancer), MCF-7 (breast cancer), HL-60 (Human promyelocytic leukemia), and HepG2 (Hepatocellular carcinoma) along with MCF 12A (normal epithelial cell) in reference to cisplatin as reference drug. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of all the cell lines HeLa, MCF-7, HL-60, HepG2, and MCF 12A. The response parameter calculated was the IC_{50} values, which responds to the concentration required for 50% inhibition of cell viability, and results are presented in Table 1.

The results as presented in Table 1, compound **7e** was revealed as the most potent compound among all the derivatives as comparable to cisplatin against HeLa cells. In the next instance, the activity was significantly reduced on replacing chloro with fluoro (**7i**) against all cell lines. The replacement of fluoro by nitro leads to further decline in the activity, **7c**. The insertion of di-nitro in the aromatic group along with replacing nitro with methyl cause a significant reduction in the activity against the tested cell lines **7a**. Compound **7j** causes further reduction of activity against HeLa cells with a similar pattern of inhibition as compound **7a** against MCF-7, HL-60, and HepG2. Compound **7d**, **7h**, and **7f** showed a further reduction in the inhibitory activity. The least to no activity was reported in the case of the remaining compound, that is, compounds **7g** and **7b**. The entire set of compounds found nontoxic to MCF-12 A normal cells.

2.3 | EGFR-TKs inhibitory activity

The highly five potent derivative from the above study viz., compounds **7a**, **7c**, **7e**, **7i**, and **7j** were evaluated *in vitro* for EGFR-TK enzyme inhibitory activity (at 10 μM) and the results as presented in Table 2 are reported as percent of inhibition in comparison to Erlotinib as a standard. The results suggested that compound **7c** (96.3%) showed the most potent inhibition of EGFR-TK followed by **7e** (90.5%). A significant decline in activity was reported in the case of compound **7i** (71.6%). The moderate inhibition was reported in the case of compound **7j**. The least activity was reported in the case of compound **7a** against EGFR-TK, which was found less than 50%, as compared to eroltinib.

2.4 | Antibacterial activity

The synthesized hybrid derivatives were tested for their *in vitro* antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus cereus*), and Gram-negative bacteria (*Pseudomonas aeruginosa*,

TABLE 3 Antibacterial activity of target hybrid derivatives **7**(**a-j**)

	Minimum inhibitory concentration (μg mL^{-1})					
Compounds	***S. aureus***	***B. subtilis***	***B. cereus***	***P. aeruginosa***	***E. coli***	***P. vulgaris***
7a	12.5	25	12.5	12.5	25	25
7b	25	50	25	12.5	25	25
7c	3.12	25	12.5	3.12	12.5	3.12
7d	50	25	25	25	50	3.12
7e	50	50	25	25	25	25
7f	3.12	25	50	25	25	50
7g	50	25	25	25	25	50
7h	25	25	25	25	25	50
7i	25	25	25	25	50	50
7j	50	12.5	50	25	25	50
Cefixime	3.12	3.12	3.12	3.12	6.25	3.12

Escherichia coli, and *Proteus vulgaris*) by the broth dilution technique in terms of MIC using Cefixime as reference as presented in Table 3. The term higher, equipotent, and moderate activity were considered for the concentration range of 3.125, 6.25–25, and 50–100 μg mL^{-1}, respectively. Compound **7a** bearing with N-methyl piperazine showed moderate activity against all the three Gram-positive (*S. aureus, B. subtilis,* and *B. cereus*) and three Gram-negative strains (*P. aeruginosa E. coli,* and *P. vulgaris*). Whereas the compound **7b** showed mild to moderate activity against all the bacterial strains, expect *B. subtilis*. The insertion of *p*-NO_2 on 1,3,5-triazine (**7c**) showed improved activity against *S. aureus, P. aeruginosa,* and *P. vulgaris* and moderate inhibitory effect against *B. subtilis, B. cereus,* and *E. coli*. However, the replacement of *para*-NO_2 with 4-methoxyaniline **7d** showed improved activity against *P. vulgaris* and found moderate activity against *B. subtilis, B. cereus,* and *P. aeruginosa* with mild activity against *S. aureus* and *E. coli*. The compound **7e,** (*p*-Cl) showed significant activity against *B. cereus, P. aeruginosa, E. coli,* and *P. vulgaris* and found moderate activity against *S. aureus* and *B. subtilis*. Whereas, the introduction of diethylamine on the 1,3,5-triazine (**7f**) showed potent activity against *S. aureus,* with mild to moderate activity against the rest of the strains. Moreover, replacing diethylamine with dimethylamine (**7g**) render compound moderately active against *B. subtilis, B. cereus, P. aeruginosa,* and *E. coli* with mild activity against *S. aureus* and *P. vulgaris*. Compound **7h** having cyclohexamine on 1,3,5-triazine showed moderate activity against all the three Gram-positive (*S. aureus, B. subtilis,* and *B. cereus*) and two Gram-negative bacterial strains (*P. aeruginosa* and *E. coli*) with mild activity against *P. vulgaris*. Compound **7i** containing *p*-fluoro showed considerable inhibitory activity against entire Gram-positive and Gram-negative strains, except *E. coli* and *P. vulgaris*. Compound **7j** bearing morpholine showed significant inhibitory activity against *B. subtilis, P. aeruginosa,* and *E. coli* and mild activity against *S. aureus, B. cereus,* and *P. vulgaris*.

TABLE 4 Antifungal activity of target hybrid derivatives **7**(**a-j**)

	Minimum inhibitory concentration (μg mL^{-1})		
Compounds	***C. albicans***	***A. niger***	***A. fumigatus***
7a	16	16	16
7b	08	08	08
7c	16	32	16
7d	08	08	16
7e	16	32	16
7f	08	32	16
7g	16	16	16
7h	08	16	08
7i	16	32	16
7j	08	08	16
Fluconazole	04	08	08

Based on these antibacterial screening results, it has been suggested that electron-withdrawing groups such as nitro and methoxy on the fourth position of the phenylamine are necessary for the potential antimicrobial compounds development.

2.5 | Antifungal activity

All the synthesized compounds **7**(**a-j**) have been evaluated for antifungal activity against *Candida albicans*, *Aspergillus niger,* and *Aspergillus fumigatus* using fluconazole as a standard drug as shown in Table 4. The term higher,

equipotent, and moderate activity were considered for the concentration range of 4.0, 8.0–16, and 32 μg mL^{-1}, respectively. The compound **7b** bearing 1,3-diaminopropane on *s*-triazine showed moderate activity against *A. niger, A. fumigates* and mild activity against *C. albicans*. On replacing, 1,3-diaminopropane with 4-methoxy (**7d**), no activity was found against fungal strains, except considerable inhibitory activity improved against *A. niger*. The compound **7h** having cyclohexamine on 1,3,5-triazine showed significant activity against *A. niger* and found moderate active against *C. albicans* and *A. fumigates*. Other compounds, such as **7a**, **7c**, **7e**, **7f**, **7g**, **7i**, and **7j**, bearing N-methyl piperazine, *para*-nitroaniline, *para*-chloroaniline, diethylamine, dimethylamine, and morpholine, respectively, showed mild to moderate activity against entire tested fungal strains.

3 | MATERIAL AND METHODS

3.1 | Chemistry

All commercially available solvents and reagents were of analytical grade and used without further purification. Melting points were determined on a Veego, MPI melting point apparatus and FTIR (2.0 cm^{-1}, flat, smooth, abex) were recorded on Perkin Elmer RX-I Spectrophotometer. ^{1}H NMR spectra were recorded in DMSO using Bruker Avance II 400 NMR and ^{13}C NMR spectra on Bruker Avance II 100 NMR spectrometer in $CDCl_3$ using TMS as internal standard. Mass spectra were obtained on VG-AUTOSPEC spectrometer equipped with electrospray ionization sources. Elemental analysis was carried out on Vario EL-III CHNOS elemental analyzer.

3.2 | Synthesis

3.2.1 | 7-chloro-4-hydrazinylquinoline (3)

A mixture of 4,7-dichloroquinoline (**1**) (0.10 mol), hydrazine hydrate (**2**) (0.15 mol) in 80 mL absolute ethanol was refluxed for 9 hours. The resulting reaction mixture was cooled and dried after filtration. The final product was recrystallized from ethanol to give compound **3**. The reaction was monitored by TLC using ethanol:acetone (1:1) as mobile phase.[26]

Yellow crystals; Yield: 84%; M.p.: 225–226°C; MW:193.63; R_f: 0.68; FTIR (ν_{max}; cm^{-1} KBr): 3267 (N—H secondary), 3272 (NH2 primary), 3053 (C—H broad), 1618 (C═C), 1549–1448 (aromatic C═N), 1263 (C—N); ^{1}H NMR (400 MHz, DMSO, TMS) δ ppm: 8.38 (d, 1H J = 5.4 Hz, quinoline), 8.25(d, 1H J = 2.5 Hz, quinoline), 7.87 (s, 1H, quinoline), 7.34 (d, 1H J = 2.3 Hz, quinoline), 6.42 (d, 1H J = 5.4 Hz, quinoline), 3.89 (br,s, 1H, NH), 1.95 (s, 2H, NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 152.8, 149.6, 149.2, 134.9, 129.5, 124.8, 121.5, 119.6, 113.2; electron spray mass spectroscopy (ES-MS) *m/z*: 194.78 $(M + H)^+$; Elemental analysis for $C_9H_8ClN_3$: Calculated: C, 55.83; H, 4.16; N, 21.70. Found: C, 55.89; H, 4.13; N, 21.74.

3.2.2 | General procedure for the synthesis of di-substituted 1,3,5-triazine derivatives 5a–j

2,4,6-Trichloro-1,3,5-triazine (**4**) (0.1 mol) was dissolved in 25 mL acetone and various distinguished amines (**a–j**) (0.2 mol) were added constantly in the above solution at 40–45°C and stirred for 6 hours followed by drop-wise addition of $NaHCO_3$ solution (0.1 mol), taking care that reaction mixture does not become acidic. The reaction was monitored by TLC using benzene:ethyl acetate (9:1) as a mobile phase. The product was filtered, washed with cold water, and recrystallized with ethanol to afford pure compounds **5a–j**.[16]

3.2.3 | General procedure for the synthesis of tri-substituted 1,3,5-triazine derivatives 6a-j

Di-substituted 1,3,5-triazine derivatives **5a-j** (0.01 mol), potassium thiocyanate (0.01 mol), and K_2CO_3 (0.01 mol) were dissolved in 1,4-dioxane. The resulting mixture was refluxed for 7–8 hours in presence of tin granules that act as a catalyst. The reaction was monitored by TLC using benzene:ethyl acetate (9:1) as a mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was purified by ethanol to afford the desired product **6a-j.**[18]

3.2.4 | General procedure for the synthesis of titled compounds 7a-j

A solution of compound **3** (0.01 mol.) and desired tri-substituted 1,3,5-triazine derivatives **6a-j** (0.01 mol.) in dry acetone was refluxed at 60–65°C for 10–11 hours. The reaction was monitored by TLC using ethanol:acetone (1:1) as a mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane, washed with brine, and dried over anhydrous Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the title compounds **7a-j**.

3.2.5 | N-(4,6-bis((4-methylpiperazin-1-yl)amino)-1,3,5-triazin-2-yl)-2-(7-chloroquinolin-4-yl) hydrazinecarbothioamide 7a

Yield: 82%; MP: 236–237°C; MW: 558.11; Rf: 0.69; FTIR (ν_{max}; cm^{-1} KBr): 3392 (N—H), 3064 (Ar C—H str), 1638, 1623 (C═N str),), 1538 (N—H bend), 1165, 982 (C—N str), 831 (C—Cl str), 828 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.64 (d, 1H, J = 5.6 Hz, Quinoline-H), 8.04 (d, 1H, J = 8.2 Hz, Quinoline-H), 7.79 (d, 1H, J = 2.6 Hz, Quinoline-H), 7.35 (d, 1H, J = 7.2 Hz, Quinoline-H), 6.78 (d, 1H, J = 5.2 Hz, Quinoline-H), 3.98 (s, 4H, 4NH), 2.67–2.36 (m, 16H, 8CH_2, piperazine), 2.28 (s, 6H, 2CH_3), 1.97 (s, 1H, N—H—N—H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.1, 176.5, 172.3, 152.8, 149.6, 149.2, 34.9, 129.5, 124.9, 121.7, 119.8, 113.1, 59.2, 55.2, 46.8; ES-MS *m/z*: 559.18 (M + 1); Elemental analysis for $C_{23}H_{32}ClN_{13}S$: Calculated: C, 49.50; H, 5.78; N, 32.63; Found: C, 49.54; H, 5.81; N, 32.61.

3.2.6 | N-(4,6-bis((3-aminopropyl) amino)-1,3,5-triazin-2-yl)-2-(7-chloroquinolin-4-yl) hydrazinecarbothioamide 7b

Yield: 75%; MP: 211–212°C; MW: 476; Rf: 0.73; FTIR (ν_{max}; cm^{-1} KBr): 3394 (N—H), 3068 (Ar C—H str), 1634, 1627 (C═N str),), 1539 (N—H bend), 1168, 987 (C—N str), 832 (C—Cl str), 832 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.63 (d, 1H, J = 5.4 Hz, Quinoline-H), 8.01 (d, 1H, J = 8.1 Hz, Quinoline-H), 7.78 (d, 1H, J = 1.9 Hz, Quinoline-H), 7.33 (d, 1H, J = 7.1 Hz, Quinoline-H), 6.76 (d, 1H, J = 5.3 Hz, Quinoline-H), 5.08 (s, 4H, 2NH_2), 3.97 (s, 4H, 4N—H), 3.37–1.86 (m, 12H, 6CH_2), 1.98 (s, 1H, N—H—N—H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.2, 165.4, 164.1, 152.8, 149.5, 149.2, 134.8, 129.5, 124.9, 121.8, 119.7, 113.1, 39.6, 31.5; ES-MS *m/z*: 477.04 (M + 1); Elemental analysis for $C_{19}H_{26}ClN_{11}S$: Calculated: C, 47.94; H, 5.51; N, 32.37; Found: C, 47.93; H, 5.53; N, 32.39.

3.2.7 | N-(4,6-bis((4-nitrophenyl)amino)-1,3,5-triazin-2-yl)-2-(7-chloroquinolin-4-yl) hydrazinecarbothioamide 7c

Yield: 67%; MP: 289–290°C; MW: 604; Rf: 0.76; FTIR (ν_{max}; cm^{-1} KBr): 3397 (N—H), 3072 (Ar C—H str), 1637, 1624 (C═N str),), 1538 (N—H bend), 1526 (NO_2 str), 1163, 989 (C—N str), 834 (C—Cl str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.67 (d, 1H, J = 5.6 Hz, Quinoline-H), 8.07 (d, 1H, J = 8.2 Hz, Quinoline-H), 8.01–6.78 (m, 8H, 8CH, Ar—H), 7.79 (d, 1H, J = 1.8 Hz, Quinoline-H), 7.35 (d, 1H, J = 7.2 Hz, Quinoline-H), 6.79 (d, 1H, J = 5.4 Hz, Quinoline-H), 3.98 (s, 4H, 4 N—H),1.97 (s, 1H, N—H—N—H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.2, 171.8, 164.2, 152.8, 149.5, 149.4, 145.1, 137.8, 134.9, 129.5, 124.9, 124.6, 121.7, 119.8, 119.2, 113.1; ES-MS *m/z*: 605.0 (M + 1); Elemental analysis for $C_{25}H_{18}ClN_{11}O_4S$: Calculated: C, 49.71; H, 3.00; N, 25.51; Found: C, 49.73; H, 3.04; N, 25.54.

3.2.8 | N-(4,6-bis((4-methoxyphenyl) amino)-1,3,5-triazin-2-yl)-2-(7-chloroquinolin-4-yl) hydrazinecarbothioamide 7d

Yield: 76%; MP: 268–269°C; MW: 574.06; Rf: 0.72; FTIR (ν_{max}; cm^{-1} KBr): 3395 (N—H), 3079 (Ar C—H str), 2985, 1634, 1628 (C═N str), 1539 (N–H bend), 1167, 985 (C—N str), 834 (C—Cl str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.64 (d, 1H, J = 5.4 Hz, Quinoline-H), 8.02 (d, 1H, J = 8.1 Hz, Quinoline-H), 7.78 (d, 1H, J = 1.9 Hz, Quinoline-H), 7.58–6.98 (m, 8H, 8CH, Ar—H), 7.32 (d, 1H, J = 7.5 Hz, Quinoline-H), 6.76 (d, 1H, J = 5.6 Hz, Quinoline-H), 3.97 (s, 4H, 4 N—H), 3.74 (s, 6H, 2OCH_3), 1.98 (s, 1H, N—H—N—H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.2, 171.8, 164.2, 153.4, 152.8, 149.6, 149.4, 134.8, 131.2, 129.5, 124.8 121.8, 121.6, 119.8, 115.1, 113.1, 55.8; ES-MS *m/z*: 575.08 (M + 1); Elemental analysis for $C_{27}H_{24}ClN_9O_2S$: Calculated: C, 56.49; H, 4.21; N, 21.96; Found: C, 56.53; H, 4.19; N, 21.99.

3.2.9 | N-(4,6-bis((4-chlorophenyl) amino)-1,3,5-triazin-2-yl)-2-(7-chloroquinolin-4-yl) hydrazinecarbothioamide 7e

Yield: 79%; MP: 276–277°C; MW: 582.89; Rf: 0.81; FTIR (ν_{max}; cm^{-1} KBr): 3394 (N—H), 3078 (Ar C—H str), 1637, 1629 (C═N str),), 1537 (N—H bend), 1169, 989 (C—N str), 835 (C—Cl str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.63 (d, 1H, J = 5.6 Hz, Quinoline-H), 8.01 (d, 1H, J = 8.2 Hz, Quinoline-H), 7.79 (d, 1H, J = 1.8 Hz, Quinoline-H), 7.67–7.24 (m, 8H, 8CH, Ar—H), 7.34 (d, 1H, J = 7.8 Hz, Quinoline-H), 6.77 (d, 1H, J = 5.4 Hz, Quinoline-H), 3.98 (s, 4H, 4 N—H), 1.97 (s, 1H, N—H—N—H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.2, 171.8, 164.2, 152.8, 149.6, 149.4, 137.2, 134.8, 129.7, 129.5, 127.8, 124.9, 122.1, 121.6, 119.8, 113.1; ES-MS *m/z*: 582.89

(M + 1); Elemental analysis for $C_{25}H_{18}Cl_3N_9S$: Calculated: C, 51.51; H, 3.11; N, 21.63; Found: C, 51.54; H, 3.12; N, 21.58.

3.2.10 | N-(4,6-bis (diethylamino)-1,3,5-triazin-2-yl)-2-(7-chloroquinolin-4-yl) hydrazinecarbothioamide 7f

Yield: 69%; MP: 249–251°C; MW: 474.03; Rf: 0.75; FT-IR (ν_{max}; cm^{-1} KBr): 3399 (N—H), 3072 (Ar C—H str), 2986, 1639, 1624 (C═N str),), 1539 (N—H bend), 1165, 986 (C—N str), 839 (C—Cl str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.64 (d, 1H, *J* = 5.7 Hz, Quinoline-H), 8.02 (d, 1H, *J* = 8.1 Hz, Quinoline-H), 7.79 (d, 1H, *J* = 1.9 Hz, Quinoline-H), 7.33 (d, 1H, *J* = 7.7 Hz, Quinoline-H), 6.76 (d, 1H, *J* = 5.4 Hz, Quinoline-H), 3.97 (s, 2H, 2 N—H), 3.26 (q, 8H, $4CH_2$), 1.97 (s, 1H, N—H—N—H), 1.23 (t, 12H, $4CH_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.1, 177.8, 171.8, 152.8, 149.6, 149.4, 134.9, 129.5, 124.8, 121.6, 119.8, 113.2, 44.6, 12.8; ES-MS *m/z*: 475.06 (M + 1); Elemental analysis for $C_{21}H_{28}ClN_9S$: Calculated: C, 53.21; H, 5.95; N, 26.59; Found: C, 53.23; H, 5.97; N, 26.57.

3.2.11 | N-(4,6-bis (dimethylamino)-1,3,5-triazin-2-yl)-2-(7-chloroquinolin-4-yl) hydrazinecarbothioamide 7g

Yield: 76%; MP: 197–198°C; MW: 417.92; Rf: 0.79; FTIR (ν_{max}; cm^{-1} KBr): 3393 (N—H), 3071 (Ar C—H str), 2987, 1636, 1628 (C═N str),), 1534 (N—H bend), 1168, 983 (C—N str), 838 (C—Cl str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.62 (d, 1H, *J* = 5.8 Hz, Quinoline-H), 8.01 (d, 1H, *J* = 8.2 Hz, Quinoline-H), 7.78 (d, 1H, *J* = 1.9 Hz, Quinoline-H), 7.34 (d, 1H, *J* = 7.9 Hz, Quinoline-H), 6.78 (d, 1H, *J* = 5.4 Hz, Quinoline-H), 3.98 (s, 2H, 2 N—H), 3.06 (s, 12H, $4CH_3$), 1.97 (s, 1H, N—H—N—H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.2, 177.8, 171.8, 152.8, 149.6, 149.4, 134.8, 129.5, 124.9, 121.6, 119.8, 113.2, 38.4; ES-MS *m/z*: 418.96 (M + 1); Elemental analysis for $C_{17}H_{20}ClN_9S$: Calculated: C, 48.86; H, 4.82; N, 30.16; Found: C, 48.83; H, 4.81; N, 30.18.

3.2.12 | N-(4,6-bis (cyclohexylamino)-1,3,5-triazin-2-yl)-2-(7-chloroquinolin-4-yl) hydrazinecarbothioamide 7h

Yield: 79%; MP: 285–287°C; MW: 526.10; Rf: 0.87; FTIR (ν_{max}; cm^{-1} KBr): 3408 (N—H), 3072 (Ar C—H str), 2985, 1638, 1629 (C═N str),), 1537 (N—H bend), 1167, 982 (C—N str), 836 (C—Cl str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.64 (d, 1H, *J* = 5.9 Hz, Quinoline-H), 8.03 (d, 1H, *J* = 8.1 Hz, Quinoline-H), 7.79 (d, 1H, *J* = 1.8 Hz, Quinoline-H), 7.32 (d, 1H, *J* = 7.8 Hz, Quinoline-H), 6.76 (d, 1H, *J* = 5.3 Hz, Quinoline-H), 3.97 (s, 4H, 4N—H), 2.58 (s, 2H, 2CH) 1.71–1.11 (m, 20H, $10CH_2$), 1.97 (s, 1H, N—H—N—H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.2, 171.8, 160.8, 152.9, 149.5, 149.4, 134.8, 129.5, 124.8, 121.6, 119.8, 113.1, 54.8, 25.7, 25.1, 32.8; ES-MS *m/z*: 527.12 (M + 1); Elemental analysis for $C_{25}H_{32}ClN_9S$: Calculated: C, 57.07; H, 6.13; N, 23.96; Found: C, 57.04; H, 6.11; N, 23.97.

3.2.13 | N-(4,6-bis((4-fluorophenyl) amino)-1,3,5-triazin-2-yl)-2-(7-chloroquinolin-4-yl) hydrazinecarbothioamide 7i

Yield: 65%; MP: 279–280°C; MW: 549.99; Rf: 0.81; FTIR (ν_{max}; cm^{-1} KBr): 3396 (N—H), 3078 (Ar C—H str), 1632, 1634 (C═N str), 1539 (N—H bend), 1208 (Ar—F), 1165, 987 (C—N str), 839 (C—Cl str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.62 (d, 1H, *J* = 5.8 Hz, Quinoline-H), 8.02 (d, 1H, *J* = 8.2 Hz, Quinoline-H), 7.78 (d, 1H, *J* = 1.9 Hz, Quinoline-H), 7.41–7.31 (m, 8H, 8CH, Ar—H), 7.34 (d, 1H, *J* = 7.7 Hz, Quinoline-H), 6.78 (d, 1H, *J* = 5.2 Hz, Quinoline-H), 3.97 (s, 4H, 4N—H), 1.98 (s, 1H, N—H—N—H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.1, 171.8, 164.2, 157.4, 152.8, 149.5, 149.4, 134.8, 134.4, 129.5, 124.9, 121.6, 120.6, 119.8, 116.4, 113.1; ES-MS *m/z*: 550.97 (M + 1); Elemental analysis for $C_{25}H_{18}ClF_2N_9S$: Calculated: C, 54.60; H, 3.30; N, 22.92; Found: C, 54.62; H, 3.28; N, 22.95.

3.2.14 | 2-(7-chloroquinolin-4-yl)-*N*-(4,6-dimorpholino-1,3,5-triazin-2-yl) hydrazinecarbothioamide7j

Yield: 77%; MP: 265–266°C; MW: 501.99; Rf: 0.83; FTIR (ν_{max}; cm^{-1} KBr): 3398 (N—H), 3079 (Ar C—H str), 1641, 1637 (C═N str),), 1535 (N—H bend), 1168, 985 (C—N str), 838 (C—Cl str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.63 (d, 1H, *J* = 5.8 Hz, Quinoline-H), 8.02 (d, 1H, *J* = 8.2 Hz, Quinoline-H), 7.79 (d, 1H, *J* = 1.8 Hz, Quinoline-H), 7.32 (d, 1H, *J* = 7.6 Hz, Quinoline-H), 6.78 (d, 1H, *J* = 5.3 Hz, Quinoline-H), 3.98 (s, 2H, 2 N—H), 3.74–3.63 (m, 16H, $8CH_2$, Morpholine), 1.98 (s, 1H, N—H—N—H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:182.2, 177.8, 171.7, 152.8, 149.5, 149.4, 134.8, 129.5, 124.8, 121.6, 119.8, 113.1, 66.4, 48.7; ES-MS *m/z*: 503.02 (M + 1); Elemental analysis for $C_{21}H_{24}ClN_9O_2S$: Calculated: C, 50.24; H, 4.82; N, 25.11; Found: C, 50.27; H, 4.82; N, 25.12.

3.3 | Anticancer evaluation

In vitro cytotoxicity was determined using a standard MTT assay with protocol appropriate for the individual test system. The four human cancer cell lines HeLa, MCF-7, HL-60, and HepG2 were cultured in the MEM medium supplemented with 10% FBS, 1% glutamine, and 50 mM/ml gentamicin sulfate in a CO_2 incubator in a humidified atmosphere of 5% CO_2 and 95% air. The cells were seeded in 96-well plates at a density of 5×10^3 cells per well and incubated until confluency 90%–95%. Then, each well was treated with 100 μL medium containing the desired concentrations of test derivatives (1–100 μM) and incubated for 48 hours. Cells in the control wells received the same volume of medium containing 0.1% DMSO. About 20 μL MTT working solution (5 mg/mL) was then added to each well and incubated for another 4 hours. At the end of incubation, the medium was carefully removed and 200 μL DMSO was added. By using ELISA reader, the optical density was measured at 540 nm. The experiment was performed in triplicate. Cell survival was calculated as the percentage of MTT inhibition as %growth inhibition = 100 − (mean OD of individual test Group/Mean OD of each Control Group) × 100.

3.4 | EGFR TK inhibitory activity

Kinase activity was determined using Kinase-Glo Plus luminescence kinase assay kit, by quantitating the amount of ATP remaining in the solution of kinase reaction.[15] The luminescent signal is correlated with the residual amount present and it was inversely related to kinase activity. The tested compounds were diluted to 100 mM in 10% DMSO, and then 5 mL of the dilution was added to a 50 mL reaction. All of the enzymatic reactions were performed at 30°C for 40 minutes, 50 mL of reaction mixture contains 10 mM $MgCl_2$, 40 mM Tris, pH 7.4, 0.1 mg/mL BSA, 0.2 mg/mL Poly (Glu, Tyr) substrate, 10 mM ATP and EGFR. Incubate the plate for 5 minutes at room temperature and then add 50 mL of Kinase-GloPlus Luminescence kinase assay to each reaction. ADP-Glo assay kit is the protein kinase assays used to determine IC_{50} values in which ADP generation was measured as it leads to an increase in luminescence signal. The reaction mixture was incubated in a 96-well plate at 30°C for 30 minutes; after the incubation period add 25 mL of ADP-Glo reagent to terminate the assay. Shake the 96-well plate for 30 minutes at ambient temperature and incubate it, then add 50 mL of kinase detection reagent. Read the 96-well plate using the ADP-Glo Luminescence reader. All the assay components were added to the blank control except the substrate. By removing the blank control value, you can obtain the corrected activity for each protein kinase target.

3.5 | Antibacterial screening

All synthesized compounds were screened for determination of their minimum inhibitory concentration (MIC, μg/mL) against selected Gram-positive organisms viz., *B. subtilis* (NCIM-2063), *B. cereus* (NCIM-2156), *S. aureus* (NCIM-2079) and Gram-negative organism viz., *P. aeruginosa* (NCIM-2036), *E. coli* (NCIM-2065), *P. vulgaris* (NCIM-2027) by the broth dilution method as recommended by the National Committee for Clinical Laboratory Standards with minor modifications.[27] Cefixime was used as a standard antibacterial agent. Solutions of the test compounds and reference drugs were prepared in dimethyl sulfoxide (DMSO) at concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL. Eight tubes were prepared in duplicate with the second set being used as MIC reference controls (16–24 hours visual). After sample preparation, the controls were placed in a 37°C incubator and read for macroscopic growth (clear or turbid) the next day. Into each tube, 0.8 mL of nutrient broth was pipetted (tubes 2–7), tube 1 (negative control) received 1.0 mL of nutrient broth and tube 8 (positive control) received 0.9 mL of nutrient. Tube 1, the negative control, did not contain bacteria or antibiotics. The positive control, tube 8, received 0.9 mL of nutrient broth because it contained bacteria but not antibiotics. The test compound was dissolved in DMSO (100 μg/mL), 0.1 mL of increasing concentration of the prepared test compounds that are serially diluted from tube 2 to tube 7 from highest (100 μg/mL) to lowest (3.125 μg/mL) concentration (tubes 2–7 containing 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL). After this process, each tube was inoculated with 0.1 mL of the bacterial suspension whose concentration corresponded to 0.5 McFarland scale (9×10^8 cells/mL) and each bacterium was incubated at 37°C for 24 hours at 150 rpm. The final volume in each tube was 1.0 mL. The incubation chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentration of the substance that gave no visible turbidity, that is, no growth of inoculated bacteria.

3.6 | Antifungal screening

All synthesized compounds were screened for determination of their MIC (μg/mL) against selected fungal strain

viz., *C. albicans*, *A. niger*, and *A. fumigates* by the broth dilution method as recommended by the National Committee for Clinical Laboratory Standards for yeasts.[28] The fungi used as inocula were grown overnight on sabouraud dextrose agar (SDA) at 25 ± 1°C. To 5 mL of SDA, 0.2 mL of culture was added and inoculated followed by incubation until it reached the turbidity equal to that of the standard 0.5 McFarland solution in 0.9% (*w*/*v*) NaCl at 600 nm, which was equivalent to 10^6–10^8 CFU/mL. A stock solution of 10 mg/mL was prepared in DMSO (Sigma) for the various synthesized compounds and for fluconazole, which were used as control. Twofold dilutions of test compounds from 64 to 02 μg/mL were prepared with the suspension of the inoculum. The micro dilution tubes, which contained 0.1 mL of the serially diluted drug, were inoculated with 0.1 mL of the resulting suspension. The final inoculum concentration after dilution with the drug suspension was 10^3/10^4 cells/mL. Two tubes containing the drug-free medium and inoculum were used as controls. The inoculated tubes were incubated at 35 ± 1°C for 48–72 hours in ambient air. The growth in each tube was then visually estimated. The MICs were determined visually and were defined as the lowest concentration of a compound that inhibits growth of the organism as detected visually.

4 | CONCLUSIONS

In the present study, a novel series of 4-aminoquinoline-1,3,5-triazines were developed as broad-spectrum antiproliferative, anti-bacterial, and anti-fungal agent. The results suggest the utility of these molecules against cancer and their co-infections, which is the major cause of mortality during the course of cancer treatment. However, more studies are needed to optimize these molecules to get potent inhibitor, and our study is in progress toward the development of new hybrid derivatives of this skeleton and will be reported in due course.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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