Accepted Manuscript

Design and synthesis of novel quinacrine-[1,3]-thiazinan-4-one hybrids for their anti-breast cancer activity

V. Raja Solomon, Sheetal Pundir, Hoang-Thanh Le, Hoyun Lee

PII: S0223-5234(17)31000-0

DOI: 10.1016/j.ejmech.2017.11.097

Reference: EJMECH 9972

To appear in: *European Journal of Medicinal Chemistry*

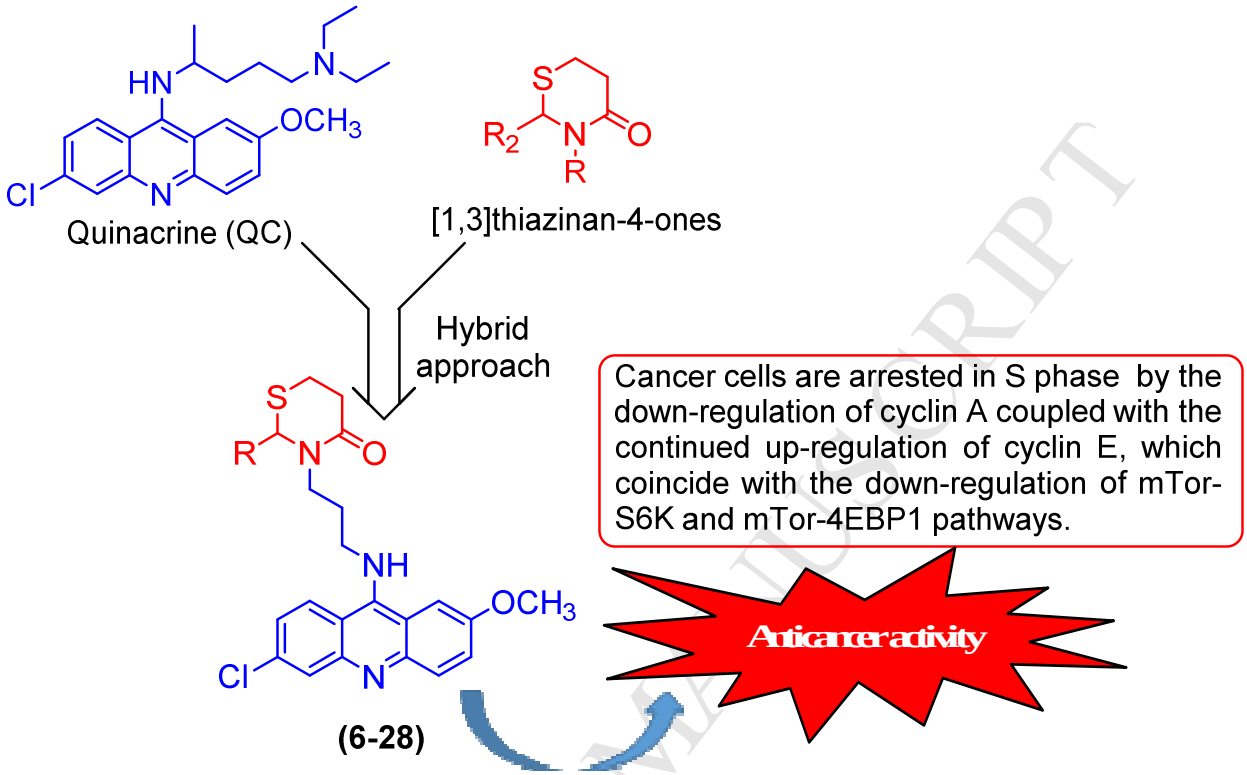
Received Date: 30 October 2017

Revised Date: 28 November 2017

Accepted Date: 29 November 2017

Please cite this article as: V.R. Solomon, S. Pundir, H.-T. Le, H. Lee, Design and synthesis of novel quinacrine-[1,3]-thiazinan-4-one hybrids for their anti-breast cancer activity, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.11.097.

Graphical Abstract



Design and synthesis of novel quinacrine-[1,3]-thiazinan-4-one hybrids for their anti-breast cancer activity

V. Raja Solomon[†,‡]*, Sheetal Pundir[†,‡], Hoang-Thanh Le[†] and Hoyun Lee[†,§,]*

[†]Health Sciences North Research Institute, 41 Ramsey Lake Road, Sudbury, Ontario P3E 5J1, Canada §Departments of Medicine, the Faculty of Medicine, the University of Ottawa, Ottawa, Ontario K1H 5M8, Canada. ‡These two authors contributed equally.

* Corresponding author Email:vrajasolomon@gmail.com; hlee@hsnri.ca

Abstract

In an attempt to develop effective and safe anticancer agents, we designed, synthesized and examined 23 novel quinacrine (QC) derivatives by combining the 9-aminoacridine scaffold and the [1,3]thiazinan-4-ones group. Most of these hybrids showed strong anticancer activities, among which 3-(3-(6-chloro-2-methoxyacridin-9-ylamino)propyl)-2-(thiophen-2-yl)-1,3-thiazinan-4-one (**25**; **VR151**) effectively killed many different cancer cell types, including eight breast cancer cell lines with different genetic background, two prostate cancer and two lung cancer cell lines. In contrast, compound **25** is less effective against non-cancer cells, suggesting it may be less toxic to humans. Our data showed that cancer cells are arrested in S phase for a prolonged period due to the down-regulation of DNA replication, leading to eventual cell death. We have also shown that the S phase arrest may be resulted by the down-regulation of cyclin A coupled with the continued up-regulation of cyclin E, which coincide with the down-regulation of mTor-S6K and mTor-4EBP1 pathways.

Keywords: 9-aminoacridine, hybrid molecules, anticancer agents, cyclins, S phase arrest, inhibition of DNA replication.

Abbreviations: DCC, *N*,*N*-dicyclohexylcarbodiimide; SRB, sulforhodamine B; QC, Quinacrine, CQ, chloroquine; CP, cisplatin; EdU, 5-ethynyl-2'-deoxyuridine; HU, hydroxyurea

1. Introduction

Breast cancer remains the most commonly diagnosed cancer among women as almost a million new cases are diagnosed globally each year [1]. Early detection, understanding of the heterogeneity of this disease, and development of targeted therapies have played a major role in reducing breast cancer mortality rates during the last few decades [2, 3]. Unfortunately, however, the clinical use of chemotherapeutics is often limited due to undesirable toxic effects [3,4] underscoring the need of new more effective and safe agents. A promising new approach to achieve this goal may be combining two or more pharmacophores into a single molecule [5]. There is evidence that a single molecule containing more than one pharmacophore, each with different mode of action, could be beneficial for the treatment of cancer [6,7]. Compounds generated by a hybrid approach can not only reduce undesirable side effects [6-8], but also often provide the opportunity of overcoming drug-resistance [9-11].

The 9-aminoacridine, the main scaffold of quinacrine (QC, Figure 1), is found in many drugs commonly used for the treatment of malaria. QC has also been reported to treat lupus erythematosus, rheumatoid arthritis, bronchial asthma and other inflammatory diseases [12, 13]. Other studies showed that QC effectively killed cancer cells by inhibiting topoisomerases, the NF-kB and Wnt-TCF signaling pathways as well as inducing p53 and p21 tumor suppressors [14-16]. Satapathy et al. reported that a hybrid nanoparticle of bioactive QC and silver substantially enhanced the cytotoxicity and reduced angiogenesis in oral cancer stem cells [17].Gomes et al. showed that novel *N*-cinnamoyl QC derivatives have substantial anti-proliferative potential against the MKN-28, Caco-2, MCF7 and HFF-1 cell lines, probably through a bimodal mechanism of DNA intercalation and interaction with grooves. We previously synthesized hybrid compounds by linking the main structural unit of the 9-aminoacridine ring system with a five membered heterocyclic ring system (VR-118, Figure 1), which showed preferential cancer-cell killing over non-cancer cells [8].

Small heterocyclic molecules have recently been recognized to have great potential for the discovery of new drug candidates. The [1,3]thiazinan-4-ones group is such a privileged pharmacophore found in many biologically active compounds. This heterocyclic system is associated with substantially greater affinity to certain anticancer targets including non-membrane protein tyrosine phosphatase (SHP-2), JNK-stimulating phosphatase-1 (JSP-1), tumor necrosis

factor TNF-α, anti-apoptotic biocomplex Bcl-XL-BH3 and integrin [19-21]. Considering these previous observations, we surmised that the hybridization of the pharmacophores 9-aminoacridine and [1,3]thiazinan-4-ones could show highly effective anticancer activity. Based on this postulation, we synthesized hybrid compounds by linking the main structural unit of the 9-aminoacridine ring system with the [1,3]thiazinan-4-ones group (Figure 1, Table 1), and examined their cytotoxic effects against three human breast tumor and one matching non-cancer cell lines (Table 1), and compound **25** (VR151) was further examined against nine other cancer cell lines and two non-cancer cell lines (Table 2).

2. Results and discussion

2. 1. Chemistry

The target compounds **6-28** were prepared as outlined in Scheme 1. The 6,9-dichloro-2-methoxyacridine (**4**) was synthesized according to the protocol described previously, in which *N*-arylanthranilic acid (**3**) was obtained from reaction between 2,4, dichlorobenzoic acid (**1**) and *p*-anisidine (**2**), followed by condensation with phosphorus oxychloride [22]. The amino components (**5**) used in the present study were prepared by aromatic nucleophilic substitution on 6,9-dichloro-2-methoxyacridine with excess of 1,3-diamino propane in neat conditions with the simple standard workup procedure reported earlier from our laboratory [23]. The quinacrine derived thiazinan-4-ones were obtained from the appropriate amine (**5**), substituted aldehyde, and mercapto propionic acid in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) in anhydrous THF at room temperature (Scheme 1, Tables 1). Here DCC was used as a dehydrating agent to accelerate the intramolecular cyclization, which was rapid and resulted in moderate to high yields [24].[26] After completion of the reaction, which usually takes 1.0 h, desired products were obtained in excellent yields and purity. Due to the formation of a new stereocenter at C-2 position, the expected enantiomeric (*2S/2R*) form were not separable by column chromatography. In the ^{1}H NMR spectra, the signals of the respective protons of the synthesized compounds were confirmed based on their chemical shifts, multiplicities and coupling constants. The compounds reported in this study have been thoroughly characterized by elemental analysis and mass spectral data.

2.2. Cell killing/anti-proliferative effects of the compounds against cancer and non-cancer cells

All the compounds synthesized were evaluated for their anti-growth/anti-proliferative effect against established tumor and non-tumor cell lines, for which compounds were diluted to achieve at least seven different concentrations ranging from 100 µM to sham control. Following incubation for 48 h in the presence of a compound, the cells were stained with sulforhodamine B (SRB) to measure the drug effects, as described previously [6, 8, 23]. The reading of SRB staining is known to accurately reflect the levels of total cellular macromolecules. The IC_{50} value of each compound was calculated with a reference to a standard curve (control cells), which represents the concentration that results in a 50% decrease in cell growth/proliferation/survival after 48 h of incubation (Table 1). QC, chloroquine (CQ) and cisplatin (CP) were included in the experiment for comparison with new compounds. The anti-proliferation/anti-growth effects measured by the SRB assay agreed well with data obtained by a clonogenic assay, indicating that the compounds effectively kill cells, not just inhibiting cell growth/proliferation (Figures S1 and S2).

Among 23 novel hybrid compounds examined (Tables 1), 14 compounds showed IC_{50} values in the range of 1.21-4.87 µM, 9 compounds 5.19-38.58 µM against MDA-MB468 cells. Against MDA-MB231 cells, 15 compounds showed IC_{50} values in the range of 0.77-4.87 µM and 8 compounds 5.36-40.08 µM. Against the MCF7 cell line, 15 compounds showed IC_{50} values in the range of 0.69-4.97 µM, and 8 compounds 5.05-39.33 µM. The differences in the IC_{50} values may be attributable to a number of factors such as the nature of substitution at the C-2 position of the [1,3]thiazinan-4-ones ring system and the genetic and biochemical background of the cell lines.

The structure-activity relationship (SAR) suggests that the modification at the quinacrine lateral side chain nitrogen atom substantially enhances anti-proliferative activity. The SAR analysis also suggests that the presence of 4-methyl (**7**) at the C-2 phenyl ring of the [1,3]thiazinan-4-ones moiety reduces the anti-proliferative activity in comparison with the unsubstituted phenyl compound (**6**). However, the introduction of 2-fluoro substitution (**8**) improved activity against MDA-MB468, MDA-MB231 and MCF7 cells. Although the introduction of 2-chloro (**12**) and 2-bromo (**17**) substitutions at the C-2 phenyl ring of the [1,3]thiazinan-4-ones moiety resulted a

decrease in the anti-proliferative activity in comparison with the 2-fluoro compound (**8**) on all breast cancer cell lines examined. These results clearly indicate that a smaller size of the halo of the group such as a 2-fluoro (**8**) group is favorable for anticancer activity. The compound having a 4-fluoro (**9**), 4-chloro (**13**) or 4-bromo (**18**) substitution at the C-2 phenyl ring of the [1,3]thiazinan-4-ones shows a significant decrease in anti-proliferative activity. The effect of a nitro substituent on the C-2 phenyl ring was apparent in compounds **21** and **22**, among which 2-nitro compound (**21**) was more active, as exhibited IC50 values of 1.21, 0.77 and 0.99 μM against MDA-MB468, MDA-MB231, and MCF7 cells, respectively. The compounds having a 4-dimethylamino (**19**) or a 4-diphenylamino (**20**) group at the C-2 phenyl ring showed improvement in anti-proliferative effects against MDA-MB468, MDA-MB231 and MCF7 cells, compared to compounds with a phenyl group (**2**).

The introduction of a heterocyclic ring system such as 1*H*-pyrrol-2-yl (**23**), furan-2-yl (**24**), thiophen-2-yl (**25**), to the C-2 position, as oppose to a phenyl ring, resulted in a substantial improvement of anti-proliferative effects against MCF7 cells. However, the introduction of pyridin-4-yl (**26**) or quinolin-4-yl (**27**) to the C-2 position, as oppose to a phenyl ring, resulted in a substantial loss of anti-proliferative effects against MDA-MB468 cells. The replacement of the C-2 phenyl ring with a cyclohexyl ring (**28**) at the [1,3]thiazinan-4-ones moiety resulted in a significant decrease in activity against MDA-MB468, MDA-MB231 and MCF7 cells.

Among this hybrid compound series, the compound **25** was particularly effective, as its IC_{50} values were 1.73, 2.80 and 0.69 μM against MDA-MB468, MDA-MB231 and MCF7 cells, respectively (Table 1). This data demonstrates that the anticancer effect of compound **25** on all three breast cancer cell lines was 8.4-fold (MDA-MB231) to 37.3-fold (MCF7) more effective than cisplatin (Table 1). Compound **25** was also substantially more effective than the parental QC, particularly for MCF7 cells which is 6-fold more effective (IC_{50} of 0.69 μM *vs* 4.19 μM).

2.3. Cancer cells, but not non-cancer cells, arrested in S phase in the presence of compound 25

Since this data indicated that compound **25** has significant potential as an anticancer agent, we extended our study to other cancer cell lines. Data from cell viability assays (Table 2; Figures S1 and S2) showed that the treatment of cells with 1-2 μM of compound **25** (VR151) effectively killed a wide range of tumor cell lines. Among them, MCF7, BT20 and BT474 mammary

carcinoma cells were the most sensitive as 1 μM of compound **25** nearly completely killed off the entire cancer cell population within 96 h post-treatment (Figure S2). The treatment of cells with 2 μM of compound **25** killed most of the cancer cell lines examined, including five other breast cancer cell lines (SkBr3, CAMA1, MDA-MB453, MDA-MB231 and MDA-MB468), two prostate cancer cell lines (LNCap and PC-3) and two lung cancer cell lines (A549 and NCI-H1975). In contrast, the MCF10A and 184B5 non-cancer cell lines required at least 6 μM of compound **25** to have the same effect (Table 2; Figure S1). Furthermore, data from clonogenic assays showed that compound **25** is clearly more effective than the parental QC at the same doses (0.39 μM and 1.5 μM) (Figure S1).

To gain a better understanding of the molecular mechanism about how compound **25** inhibits cell proliferation, we carried out flow cytometry to examine its effects on cell cycle progression. As shown in Figure 2A, most of the MCF7 and MDA-MB231 cell populations appeared arrested in S phase in the presence of 2 μM compound **25**, along with portion of the cell population showing sub-G1 DNA content that is typically shown with cells dying by apoptosis. This conclusion was confirmed by BrdU pulse-labeling, which showed that DNA synthesis was dramatically down-regulated as early as 6 h and essentially stopped by 48 post-treatment with 2 μM of compound **25** (Figure 2B). In contrast, the cell cycle progression of 184B5 non-cancer cells was not affected by 2 μM of compound **25** (Figure 2A).

2.4. The treatment of cancer cells with compound 25 resulted in the up-regulation of cyclin E and down-regulation of cyclin A, leading to the inhibition of DNA replication

Since data in Figure 3 indicated that cancer cells in S phase did not replicate their DNA, we examined it further by labeling two cancer cell lines and one non-cancer cell line with EdU (5-ethynyl-2'-deoxyuridine). As shown in Figure 3, approximately 10% of MDA-MB231 and 5% of MCF7 cells were EdU positive in the presence of 2 μM of compound **25**. This is a dramatic decrease of EdU incorporation into their DNA in the two cancer cell lines in the presence of compound **25**, as 45-63% of sham controls were EdU positive. As expected, the level of EdU incorporation in the 184B5 cell line was similar between the sham control (55%) and those treated with 2 μM compound **25** (52%).

To gain insight into the mechanism of the down-regulation of DNA replication, we examined the levels of cyclins since they are the main regulators of cell cycle progression [25]. Cyclin D is part of the cell cycle engine that facilitates cell cycle progression in early- to mid-G1. The Cdk2-cyclin E then takes over the role in late G1, and facilitates cell cycle progression into S phase. In S phase, the up-regulation of cyclin A and the down-regulation of cyclin E are required for the replication of both DNA and centrosome [26], while cyclin B is necessary for Cdk1 kinase function at the G2/M transition [27]. As shown in Figure 3B, the level of cyclin E was substantially elevated while that of cyclin A was extremely low by 24 h in the presence of compound **25**. On the other hand, the level of cyclin D was similar between sham-treated and treated with 2 μM compound **25**. Together, these data indicate that cell cycle progressed normally from G1 to S phase; however, the failure of cyclin A induction coupled with the elevated level of cyclin E in S phase resulted in the suppression of DNA replication. Since cells did not reach to the G2/M border, the level of cyclin B was still very low.

2.5. Compound 25 did not damage DNA

Since cancer cells arrested in S phase due to the defect in DNA replication, we examined if it was caused by DNA damage mediated by compound **25**. Unlike the X-ray control sample, the levels of H2A.x phosphorylation on Ser139 were similar between sham controls and those treated with compound **25**. This data indicates that compound **25** did not cause any notable DNA damage (Figure 4A). We then examined the activation (i.e., phosphorylation) of Chk1 and Chk2, both of which are relevant to the DNA damage-induced checkpoint control mechanisms [28]. We found that neither Chk 1 nor Chk2 was activated in the presence of compound **25** (Figure 4B). Therefore, we have concluded that the S phase arrest in the presence of compound **25** was not caused by DNA damage.

2.6. The mTor-4EBP1 and mTOR-p70/p85^{S6K} pathways were down-regulated in the presence of compound 25

Since the parental QC compound is known to inhibit mTor pathway [29], we examined whether compound **25** also inhibits the mTor pathway. We found that the phosphorylation of mTor on the Ser2448 residue was down-regulated by 6 h in the presence of 2 μM of compound **25** in MCF7 cells (Figure 5A). Although the treatment of compound **25** did not result in any alteration of S6K

phosphorylation on Thr389 by 6 h post-treatment, it did markedly down-regulate the phosphorylation by 24 h post-treatment (Figure 5B). Interestingly, however, phosphorylation of S6K on Ser371 was not affected under the same conditions. Unlike rapamycin, the phosphorylation of 4EBP1 on Thr37/Thr46 was substantially down-regulated by 6 h post-treatment with compound **25** (Figure 5C). Thus, our data showed that the down-regulation of the two major downstream mTor pathways was sequential: the mTor-4EBP1 was affected early (6 h) and the mTor-S6K pathway was affected later time (24 h) in the presence of compound **25**. In contrast, the phosphorylation of Akt on Thr308 was up-regulated in the presence of compound **25** (Figure 5D), which is unexpected since the mTor pathway is considered to be the downstream of the PI3K-Akt pathway [30].[32] This point is further discussed below.

The mTor pathway regulates the translation of specific mRNA through the activation of 4EBP1 and p70/p85S6K, its two major downstream sub-pathways. The phosphorylation of 4EBP1 on Thr37 and Thr46 by mTor leads to its dissociation from eIF4E, resulting in the down-regulation of protein synthesis. The activation of S6K by mTor recruits the 40S ribosomal subunit to actively translating polysomes on mRNA. Compound **25** appears to inhibit this important molecular process essential for cell growth and survival. Unlike QC [28, 29], the Akt pathway was not down-regulated by compound **25**. This may indicate that compound **25** functions directly to mTor, particularly to the mTor-4E-BP1 pathway. It is conceivable that a cell tries (but fails) to restore the mTor pathway by up-regulating Akt activities through the feedback control mechanism [30]. Our data is thus consistent with the notion that the levels of certain proteins including cyclin A are severely down-regulated by the mTor-mediated translational down-regulation in the presence of compound **25**. However, it is currently unclear whether cyclin A is the direct target of compound **25**.

3. Conclusion

Here we describe the design, synthesis, and examination of 23 quinacrine derivatives generated by the hybridization of the QC core scaffold and [1,3]thiazinan-4-ones in an attempt to develop effective and safe anticancer agents. Most of the novel 23 hybrid compounds exhibited substantial anticancer activity against three different human breast cancer cell lines, MDA-MB468, MDA-MB231 and MCF7. Compound **25** (3-(3-(6-chloro-2-methoxyacridin-9-

ylamino)propyl)-2-(thiophen-2-yl)-1,3-thiazinan-4-one, **VR151**) was further characterized as it was the most active compound among this series. In addition to the three breast cancer cell lines, 1-2 μM of compound **25** effectively killed off all of the cancer cell lines examined including five other breast cancer cell lines with different genetic background (BT20, BT474, SkBr3, CAMA1 and MDA-MB453), two prostate cancer cell lines (LNCap and PC-3) and two lung cancer cell lines (A549 and NCI-H1975). Unlike cisplatin and the parental QC, compound **25** showed preferential killing activity against cancer over non-cancer cells: at least 3-6-fold higher concentrations of compound **25** were required to kill off the MCF10A and 184B5 non-cancer cells (i.e., 1-2 μM vs >6 μM). It should also be noted that compound **25** kills cancer cells more effective than the parental QC at the same doses.

Cancer cells progressed normally from G1 to S phase in the presence of compound **25**; however, they arrested in S phase for a prolonged time, leading to eventual cell death. The S phase arrest was not due to DNA damage, but because of defect in DNA replication. Our data is consistent with the notion that the defect in DNA replication is caused by the down-regulation of cyclin A coupled with the continued up-regulation of cyclin E during S phase. The deregulation of the cyclins A and E may be due to the down-regulation of mTor-S6K and mTor-4EBP1 pathways.

4. Materials and Methods

General

Melting points (mp) were taken in open capillaries on the Complab melting point apparatus. Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values were within the acceptable limit (±1.5%) of the calculated values. The ^{1}H spectra were recorded on a DPX-500 MHz Bruker FT-NMR spectrometer using $CDCl_3$ and DMSO-d_6 as solvent. The chemical shifts were reported as parts per million (δ ppm) tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). The progress of the reaction was monitored on ready-made silica-gel plates (Merck) using chloroform-methanol (9:1) as solvent. Iodine was used as a developing agent or by spraying with the Dragendorff's reagent. Chromatographic purification was

performed over a silica gel (100-200 mesh). The residues were obtained recrystallized by the addition of 80:20 hexane–chloroform. All chemicals and reagents obtained from Aldrich (USA) were used without further purification.

4-Chloro-2-(phenylamino)benzoic acid (3)

4-Chloro-2-(phenylamino)benzoic acid was synthesized by the condensation of 2,4, dichlorobenzoic acid (**1**) and *p*-anisidine (**2**) in the presence of $LiNH_2$ as reported [22].[22] (Scheme 1).

6,9-Dichloro-2-methoxyacridine (4)

6,9-Dichloro-2-methoxyacridine was synthesized by the cyclisation of 4-chloro-2-(phenylamino)benzoic acid with phosporus oxychloride as reported [22] (Scheme 1).

***N*^{1}-(6-Chloro-2-methoxy-acridin-9-yl)-propane-1,3-diamine (5)**

The mixture of 6,9-dichloro-2-methoxyacridine (20.25 mmol), 1,3-diamino propane (1.96 ml, 25.75 mmol) and triethylamine (3.6 ml, 25.75 mmol) was heated slowly to 80 ºC for longer than 1 h while stirring. The temperature was then increased to 130-140 ºC, where it was kept for 6 h while stirring continuously. The reaction mixture was cooled to room temperature, and then poured into ice-cold water and filtered. The precipitate was filtered, washed, and recrystallized using chloroform: methanol (3:1) mixture to obtain as cream-yellow solid. Yield 85%; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.81-1.85 (m, 2H, CH_2), 2.76-2.79 (m, 2H, CH_2), 2.89-2.91 (m, 2H, CH_2), 3.25 (br s, 2H, N*H*$_2$ D_2O-exchangeable), 3.87 (s, 3H, OCH_3), 6.98 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.11 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.18 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.47 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.81 (br s, 1H, N*H* D_2O-exchangeable), 7.96 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.19 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 32.25 (2C), 49.74 (2C), 55.65 (2C), 100.67, 114.62, 117.03, 122.67, 122.97, 130.54, 134.11, 146.09, 148.68, 150.75, 155.37; ES-MS *m/z* 315 (M+, 100), 317

(M+2, 54); Anal.Calcd for $C_{17}H_{18}ClN_3O$: C, 64.66; H, 5.75; N, 13.31; Found: C, 64.61; H, 5.63; N, 12.96.

General synthetic procedure for compounds (6-28)

The amino component (1.0 mmol) and aldehyde (2.0 mmol) were stirred in THF under ice-cold conditions for 5 min, followed by addition of the mercapto propionic acid component (3.0 mmol). After 5 min, DCC (1.2 mmol) was added to the reaction mixture at 0 °C and the reaction mixture was stirred for an additional 50 min at room temp. DCU was removed by filtration, the filtrate was concentrated to dryness under reduced pressure, and the residue was taken up in chloroform. The organic layer was successively washed with 5% aq. sodium hydrogen carbonate and then finally with brine. The organic layer was dried over sodium sulfate, and the solvent was removed under reduced pressure to get a crude product that was purified by column chromatography on silica gel using chloroform: methanol (9:1).

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-phenyl-[1,3]thiazinan-4-one (6)

Yellow solid; Yield 75%; mp 146-148 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.86-1.89 (m, 2H, CH_2), 2.90-2.97 (m, 4H, CH_2), 3.49-3.51 (m, 2H, CH_2), 3.74-3.77 (m, 2H, CH_2), 4.01 (s, 3H, OCH_3), 5.51 (s, 1H, SC*H*N), 6.53 (br s, 1H, N*H* D_2O-exchangeable), 7.25-7.28 (m, 3H, Ar-*H*), 7.35-7.42 (m, 4H, Ar-*H*), 7.55 (s, 1H, Ar-*H*), 7.96 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.04 (s, 1H, Ar-*H*), 8.09 (d, *J* = 10.0 Hz, 2H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 28.53, 34.34, 44.41, 46.07, 55.76, 61.81, 99.51, 115.49, 118.03, 123.47, 124.08, 124.85, 124.90 (2C), 126.47, 128.14, 128.80 (2C), 130.89, 131.38, 132.47, 134.63, 138.67, 150.67, 156.12, 170.71; ES-MS *m/z* 492 (M+, 100), 494 (M+2, 65); Anal.Calcd for $C_{27}H_{26}ClN_3O_2S$: C, 65.91; H, 5.33; N, 8.54; Found: C, 65.89; H, 5.38; N, 8.50.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-*p*-tolyl-[1,3]thiazinan-4-one (7)

Orange yellow solid; Yield 78%; mp 171-173 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82-1.86 (m, 2H, CH_2), 2.37 (s, 3H, CH_3), 2.84-2.95 (m, 4H, CH_2), 3.49-3.52 (m, 2H, CH_2), 3.69-3.72 (m, 2H, CH_2), 4.01 (s, 3H, OCH_3), 5.47 (s, 1H, SC*H*N), 6.41 (br s, 1H, N*H* D_2O-exchangeable), 7.13 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.19 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.26-7.28 (m, 2H, Ar-*H*), 7.39 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.55 (s, 1H, Ar-*H*), 7.96 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.05 (s, 1H, Ar-*H*), 8.10 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 506 (M+, 100), 508 (M+2, 44); Anal.Calcd for $C_{28}H_{28}ClN_3O_2S$: C, 66.45; H, 5.58; N, 8.30; Found: C, 66.52; H, 5.49; N, 8.36.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(2-fluoro-phenyl)-[1,3]thiazinan-4-one (8)

Yellow solid; Yield 68%; mp 151-153 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82-1.89 (m, 2H, CH_2), 2.79-2.84 (m, 2H, CH_2), 2.89-2.93 (m, 2H, CH_2), 2.95-2.99 (m, 2H, CH_2), 3.48-3.51 (m, 2H, CH_2), 3.99 (s, 3H, OCH_3), 5.73 (s, 1H, SC*H*N), 6.42 (br s, 1H, N*H* D_2O-exchangeable), 7.07-7.24 (m, 3H, Ar-*H*), 7.28 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.32 (dd, J_1 = 5.0 Hz, J_2 = 10.0 Hz, 1H, Ar-*H*), 7.36 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.49 (s, 1H, Ar-*H*), 7.94-(d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.02 (s, 1H, Ar-*H*), 8.05 (d, *J* = 5.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 510 (M+, 100), 512 (M+2, 44); Anal.Calcd for $C_{27}H_{25}ClFN_3O_2S$: C, 63.58; H, 4.94; N, 8.24; Found: C, 63.62; H, 5.01; N, 8.21.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(4-fluoro-phenyl)-[1,3]thiazinan-4-one (9)

Orange yellow solid; Yield 75%; mp 121-122 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.86-1.90 (m, 2H, CH_2), 2.82-7.97 (m, 4H, CH_2), 3.51-3.54 (m, 2H, CH_2), 3.83-3.91 (m, 2H, CH_2), 4.03 (s, 3H, OCH_3), 5.49 (s, 1H, SC*H*N), 6.46 (br s, 1H, N*H* D_2O-exchangeable), 7.08 (dd, J_1 = 5.0 Hz, J_2 = 10.0 Hz, 2H, Ar-*H*), 7.23 (dd, J_1= 5.0 Hz, J_2 = 10.0 Hz, 2H, Ar-*H*), 7.30-7.31 (d, *J*= 5.0 Hz, 1H, Ar-*H*), 7.41 (dd, J_1= 5.0 Hz, J_2 = 10.0 Hz, 1H, Ar-*H*), 7.55 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.98 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.06 (s, 1H, Ar-*H*), 8.12 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 510 (M+, 100), 512 (M+2, 48); Anal.Calcd for $C_{27}H_{25}ClFN_3O_2S$: C, 63.58; H, 4.94; N, 8.24; Found: C, 63.62; H, 4.91; N, 8.27.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(2,4-difluoro-phenyl)-[1,3]thiazinan-4-one (10)

Yellow solid; Yield 72%; mp 154-156 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82-1.86 (m, 2H, CH_2), 2.77-2.82 (m, 2H, CH_2), 2.88-2.92 (m, 2H, CH_2), 3.50-3.53 (m, 2H, CH_2), 3.76-3.82 (m, 2H, CH_2), 4.02 (s, 3H, OCH_3), 5.70 (s, 1H, SC*H*N), 6.33 (br s, 1H, N*H* D_2O-exchangeable), 6.91-6.94 (m, 2H, Ar-*H*), 7.07 (dd, J_1= 5.0 Hz, J_2= 10.0 Hz, 1H, Ar-*H*), 7.28 (d, *J*= 10.0 Hz, 1H, Ar-*H*), 7.40 (d, *J*= 10.0 Hz, 1H, Ar-*H*), 7.54 (s, 1H, Ar-*H*), 7.97 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.06 (s, 1H, Ar-*H*), 8.11 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 25.61, 30.33, 34.99, 44.15, 46.04, 55.06, 55.76, 99.32, 105.52, 105.22, 105.42, 124.70, 124.74, 127.51, 128.12, 131.24, 134.65, 135.81, 146.63, 148.48, 150.31, 156.14, 158.67, 163.94, 164.04, 162.04, 170.54; ES-MS *m/z* 528 (M+, 100), 530 (M+2, 52); Anal.Calcd for $C_{27}H_{24}ClF_2N_3O_2S$: C, 61.42; H, 4.58; N, 7.96; Found: C, 61.36; H, 4.62; N, 7.92.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(2,6-difluoro-phenyl)-[1,3]thiazinan-4-one (11)

Yellow solid; Yield 70%; mp 198-199 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82-1.86 (m, 2H, CH_2), 2.81-2.84 (m, 2H, CH_2), 2.95-3.02 (m, 2H, CH_2), 3.13-3.17 (m, 2H, CH_2), 3.50-3.54 (m, 2H, CH_2), 3.99 (s, 3H, OCH_3), 5.75 (s, 1H, SC*H*N), 6.44 (br s, 1H, N*H* D_2O-exchangeable), 6.91-6.98 (m, 3H, Ar-*H*), 7.24 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.36 (d, *J*= 10.0 Hz, 1H, Ar-*H*), 7.55 (s, 1H, Ar-*H*), 7.93 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.01 (s, 1H, Ar-*H*), 8.08 (d, *J* = 5.0 Hz, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 23.26, 28.22, 34.48, 44.21, 46.00, 53.43, 55.74, 99.36, 112.36, 112.39, 112.54, 115.74,. 115.78, 118.25, 123.95, 124.74, 124.93, 124.93, 127.99, 130.20, 131.09, 134.53, 148.59, 150.42, 156.08, 161.18, 170.44; ES-MS *m/z* 528 (M+, 100), 530 (M+2, 54); Anal.Calcd for $C_{27}H_{24}ClF_2N_3O_2S$: C, 61.42; H, 4.58; N, 7.96; Found: C, 61.38: H, 4.57; N, 8.01

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(2-chloro-phenyl)-[1,3]thiazinan-4-one (12)

Yellow solid; Yield 65%; mp 118-119 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.88-1.93 (m, 2H, CH_2), 2.51-2.68 (m, 4H, CH_2), 3.59-3.62 (m, 2H, CH_2), 3.75-5.78 (m, 2H, CH_2), 3.90 (s, 3H, OCH_3), 5.55 (s, 1H, SC*H*N), 6.64 (br s, 1H, N*H* D_2O-exchangeable), 7.09 (d, *J* = 10.0 Hz, 2H, Ar-*H*), 7.17 (d, *J*= 5.0 Hz, 1H, Ar-*H*), 7.28 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.32 (s, 1H, Ar-*H*), 7.53 (s, 1H, Ar-*H*), 7.89 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.84 (s, 1H, Ar-*H*), 8.13 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 526 (M+, 100), 528 (M+2, 78); Anal.Calcd for $C_{27}H_{25}Cl_2N_3O_2S$: C, 61.60; H, 4.79; N, 7.98; Found; C, 61.56; H, 4.68; N, 8.03.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(4-chloro-phenyl)-[1,3]thiazinan-4-one (13)

Orange yellow solid; Yield 67%; mp 150-152 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.87-1.92 (m, 2H, CH_2), 2.80-2.97 (m, 4H, CH_2), 3.50-3.53 (m, 2H, CH_2), 3.81-3.84 (m, 2H, CH_2), 3.90 (s, 3H, OCH_3), 5.46 (s, 1H, SC*H*N), 6.39 (br s, 1H, N*H* D_2O-exchangeable), 7.19 (d, *J* = 10.0 Hz, 2H, Ar-*H*), 7.26 (d, *J*= 5.0 Hz, 1H, Ar-*H*), 7.37-7.41 (m, 3H, Ar-*H*), 7.53 (s, 1H, Ar-*H*), 7.96 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.04 (s, 1H, Ar-*H*), 8.09 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 21.78, 28.54, 34.26, 44.43, 46.04, 61.25, 99.40, 115.68, 118.61, 124.16 (2C), 124.80, 127.81 (2C), 128.99, 129.46 (2C), 130.94, 131.30, 134.66, 134.78, 137.33 (2C), 148.26, 150.45, 156.14, 170.54; ES-MS *m/z* 526 (M+, 100), 528 (M+2, 76); Anal.Calcd for $C_{27}H_{25}Cl_2N_3O_2S$: C, 61.60; H, 4.79; N, 7.98; Found: C, 61.58; H, 4.81; N, 7.91.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(2,4-dichloro-phenyl)-[1,3]thiazinan-4-one (14)

Orange yellow solid; Yield 72%; mp 146-148 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.94-1.98 (m, 2H, CH_2), 2.70-2.77 (m, 2H, CH_2), 2.83-2.88 (m, 2H, CH_2), 3.00-3.03 (m, 2H, CH_2), 3.85-3.88 (m, 2H, CH_2), 4.02 (s, 3H, OCH_3), 5.74 (s, 1H, SC*H*N), 6.57 (br s, 1H, N*H* D_2O-exchangeable), 7.06 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.26 (s, 1H, Ar-*H*), 7.31 (d, *J*= 10.0 Hz, 1H, Ar-*H*), 7.38-7.40 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.50 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.98 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.05 (s, 1H, Ar-*H*), 8.08 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 21.22, 28.37, 34.38, 44.37,

46.01, 55.75, 58.21, 99.49, 124.22, 124.77 (2C), 124.96 (2C), 126.97 (2C), 127.17 (2C), 130.82, 133.55 (2C), 134.18 (2C), 135.06 (2C), 150.68, 156.16, 170.61; ES-MS *m/z* 561 (M+, 100), 563 (M+2, 90); Anal.Calcd for $C_{27}H_{24}Cl_3N_3O_2S$: C, 57.81; H, 4.31; N, 7.49; Found: C, 57.77; H, 4.27; N, 7.55.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(2,6-dichloro-phenyl)-[1,3]thiazinan-4-one (15)

Yellow solid; Yield 65%; mp 124-125 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.81-1.84 (m, 2H, CH_2), 2.22-2.25 (m, 2H, CH_2), 3.00-3.07 (m, 2H, CH_2), 3.11-.15 (m, 2H, CH_2), 3.98-4.02 (m, 2H, CH_2), 4.04 (s, 3H, OCH_3), 5.84 (s, 1H, SC*H*N), 6.33 (br s, 1H, N*H* D_2O-exchangeable), 7.04 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.23-7.37 (m, 2H, Ar-*H*), 7.56 (s, 1H, Ar-*H*), 7.95-7.98 (m, 2H, Ar-*H*), 8.02 (d, *J*= 10.0 Hz, 1H, Ar-*H*), 8.11 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 8.48 (s, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 24.11, 31.63, 35.37, 42.70, 46.25, 50.23, 55.32, 99.42, 100.21, 123.94, 124.07, 124.49, 124.69, 124.87, 128.79 (2C), 130.44 (2C), 132.13, 132.60, 133.57, 134.61, 136.84, 155.60, 156.15, 157.82, 170.90; ES-MS *m/z* 561 (M+, 100), 563 (M+2, 78); Anal.Calcd for $C_{27}H_{24}Cl_3N_3O_2S$: C, 57.81; H, 4.31; N, 7.49; Found: C, 57.77; H, 4.26; N, 7.52.

2-(2-Chloro-6-fluoro-phenyl)-3-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-[1,3]thiazinan-4-one (16)

Orange yellow solid; Yield 72%; mp 132-134 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82-1.91 (m, 2H, CH_2), 2.98-3.02 (m, 4H, CH_2), 3.14-3.16 (m, 2H, CH_2), 3.52-3.56 (m, 2H, CH_2), 4.03 (s, 3H, OCH_3), 5.92 (s, 1H, SC*H*N), 6.53 (br s, 1H, N*H* D_2O-exchangeable), 7.04 (m, 2H, Ar-*H*), 7.26-7.29 (m, 2H, Ar-*H*), 7.39 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.58 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.97 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.05 (s, 1H, Ar-*H*), 8.12 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 545 (M+, 100), 547 (M+2, 62); Anal.Calcd for $C_{27}H_{24}Cl_2FN_3O_2S$: C, 59.56; H, 4.44; N, 7.72; Found: C, 59.63; H, 4.28; N, 7.66.

2-(2-Bromo-phenyl)-3-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-[1,3]thiazinan-4-one (17)

Yellow solid; Yield 70%; mp 137-139 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.90-1.96 (m, 2H, CH_2), 2.71-2.77 (m, 2H, CH_2), 2.88-2.91 (m, 2H, CH_2), 3.01-3.04 (m, 2H, CH_2), 3.52-3.56 (m, 2H, CH_2), 4.03 (s, 3H, OCH_3), 5.77 (s, 1H, SC*H*N), 6.47 (br s, 1H, N*H* D_2O-exchangeable), 7.12 (dd, J_1 = 5.0 Hz, J_2 = 10.0 Hz, 1H, Ar-*H*), 7.23 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.36-7.37 (d, *J*= 5.0 Hz, 1H, Ar-*H*), 7.39 (dd, J_1= 5.0 Hz, J_2 = 10.0 Hz, 1H, Ar-*H*), 7.42 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.54-7.55 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.66 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.98 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.06 (s, 1H, Ar-*H*), 8.12 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 571 (M+, 100), 573 (M+2, 42); Anal.Calcd for $C_{27}H_{25}BrClN_3O_2S$: C, 56.80; H, 4.41; N, 7.36; Found: C, 56.72; H, 4.39; N, 7.29.

2-(4-Bromo-phenyl)-3-[3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl]-[1,3]thiazinan-4-one (18)

Orange yellow solid; Yield 69%; mp 123-124 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.63-1.78 (m, 2H, CH_2), 2.71-2.74 (m, 2H, CH_2), 2.80-2.89 (m, 2H, CH_2), 2.93-2.97 (m, 2H, CH_2), 3.52-3.56 (m, 2H, CH_2), 4.02 (s, 3H, OCH_3), 5.45 (s, 1H, SC*H*N), 6.42 (br s, 1H, N*H* D_2O-exchangeable), 7.13 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.18 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.30-7.34 (m, 2H, Ar-*H*), 7.41 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.53 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.71 (s, 1H, Ar-*H*), 7.98 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.06 (s, 1H, Ar-*H*), 8.12 (d, *J* = 5.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 571 (M+, 100), 573 (M+2, 40); Anal.Calcd for $C_{27}H_{25}BrClN_3O_2S$: C, 56.80; H, 4.41; N, 7.36; Found: C, 56.69; H, 4.50; N, 7.32.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(4-dimethylamino-phenyl)-[1,3]thiazinan-4-one (19)

Orange yellow solid; Yield 72%; mp 133-134 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.86-1.90 (m, 2H, CH_2), 2.72-2.76 (m, 2H, CH_2), 2.91-2.97 (m, 2H, CH_2), 2.98 (s, 3H, $N(CH_3)_2$), 3.57-3.61 (m, 2H, CH_2), 3.88-3.92 (m, 2H, CH_2), 4.04 (s, 3H, OCH_3), 5.51 (s, 1H, SC*H*N), 6.45 (br s, 1H, N*H*

D_2O-exchangeable), 6.70 (d, *J* = 10.0 Hz, 2H, Ar-*H*), 7.13 (d, *J* = 10.0 Hz, 2H, Ar-*H*), 7.29 (s, 1H, Ar-*H*), 7.39 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.59 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.97 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.04 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 8.13 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 536 (M+, 100), 538 (M+2, 44); Anal.Calcd for $C_{29}H_{31}ClN_4O_2S$: C, 65.09; H, 5.84; N, 10.47; Found: C, 65.16; H, 5.92; N, 10.43.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(4-diphenylamino-phenyl)-[1,3]thiazinan-4-one (20)

Orange yellow solid; Yield 70%; mp 152-154 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.88-1.92 (m, 2H, CH_2), 2.74-2.78 (m, 2H, CH_2), 2.93-2.99 (m, 2H, CH_2), 2.99 (s, 3H, $N(CH_3)_2$), 3.59-3.63 (m, 2H, CH_2), 3.90-3.95 (m, 2H, CH_2), 4.01 (s, 3H, OCH_3), 5.53 (s, 1H, SC*H*N), 6.47 (br s, 1H, N*H* D_2O-exchangeable), 6.70 (d, *J* = 10.0 Hz, 2H, Ar-*H*), 7.01-7.11 (m, 10H, Ar-*H*), 7.13 (d, *J* = 10.0 Hz, 2H, Ar-*H*), 7.29 (s, 1H, Ar-*H*), 7.39 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.59 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.97 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.04 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 8.13 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 660 (M+, 100), 662 (M+2, 38); Anal.Calcd for $C_{39}H_{35}ClN_4O_2S$: C, 71.05; H, 5.35; N, 8.50; Found: C, 71.11; H, 5.41; N, 8.58.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(2-nitro-phenyl)-[1,3]thiazinan-4-one (21)

Orange yellow solid; Yield 67%; mp 137-139 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.83-1.93 (m, 2H, CH_2), 2.66-2.75 (m, 2H, CH_2), 3.52-3.56 (m, 2H, CH_2), 3.79-3.83 (m, 2H, CH_2), 4.02 (s, 3H, OCH_3), 6.29 (s, 1H, SC*H*N), 6.52 (br s, 1H, N*H* D_2O-exchangeable), 7.12 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.28 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.37 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.41 (s, 1H, Ar-*H*), 7.55 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.61 (s, 1H, Ar-*H*), 7.63 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.67 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.74 (s, 1H, Ar-*H*), 7.75 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 538 (M+, 100), 540 (M+2, 44); Anal.Calcd for $C_{27}H_{25}ClN_4O_4S$: C, 60.39; H, 4.69; N, 10.43; Found: C, 60.42; H, 4.72; N, 10.48.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-(4-nitro-phenyl)-[1,3]thiazinan-4-one (22)

Orange yellow solid; Yield 67%; mp 137-138 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.86-1.93 (m, 2H, CH_2), 2.76-2.81 (m, 2H, CH_2), 2.91-3.00 (m, 2H, CH_2), 3.52-3.56 (m, 2H, CH_2), 3.79-3.84 (m, 2H, CH_2), 4.02 (s, 3H, OCH_3), 5.22 (s, 1H, SC*H*N), 6.25 (br s, 1H, N*H* D_2O-exchangeable), 7.28 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.42 (m, 2H, Ar-*H*), 7.97 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.05 (s, 1H, Ar-*H*), 8.09 (d, *J* = 10.0 Hz, 2H, Ar-*H*), 8.25 (d, *J* = 10.0 Hz, 2H, Ar-*H*); ES-MS *m/z* 538 (M+, 100), 540 (M+2, 48); Anal.Calcd for $C_{27}H_{25}ClN_4O_4S$: C, 60.39; H, 4.69; N, 10.43; Found: C, 60.41; H, 4.75; N, 10.49.

3-(3-(6-chloro-2-methoxyacridin-9-ylamino)propyl)-2-(1*H*-pyrrol-2-yl)-1,3-thiazinan-4-one (23)

Orange Yellow solid; Yield 60%; mp 141-143 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.87-1.91 (m, 2H, CH_2), 2.81-2.83 (m, 2H, CH_2), 2.90-2.93 (m, 2H, CH_2), 3.13-3.17 (m, 2H, CH_2), 3.89-3.93 (m, 2H, CH_2), 4.02 (s, 3H, OCH_3), 5.51 (s, 1H, SC*H*N), 6.31 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 6.39 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 6.43 (br s, 1H, N*H* D_2O-exchangeable), 6.55 (br s, 1H, N*H* D_2O-exchangeable), 7.30 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.31 (s, 1H, Ar-*H*), 7.38-7.41 (m, 2H, Ar-*H*), 7.57 (s, 1H, Ar-*H*), 7.99 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.07 (s, 1H, Ar-*H*), 8.13 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 482 (M+, 100), 484 (M+2, 48); Anal.Calcd for $C_{25}H_{25}ClN_4O_3S$: C, 62.42; H, 5.24; N, 11.65; Found: C, 62.39; H, 5.21; N, 11.69.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-furan-2-yl-[1,3]thiazinan-4-one (24)

Yellow solid; Yield 60%; mp 146-147 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.85-1.89 (m, 2H, CH_2), 2.80-2.84 (m, 2H, CH_2), 2.91-2.94 (m, 2H, CH_2), 3.12-3.16 (m, 2H, CH_2), 3.88-3.92 (m, 2H, CH_2), 4.01 (s, 3H, OCH_3), 5.49 (s, 1H, SC*H*N), 6.29-6.30 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 6.38 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 6.42 (br s, 1H, N*H* D_2O-exchangeable), 7.29 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.30 (s, 1H, Ar-*H*), 7.40-7.43 (m, 2H, Ar-*H*), 7.56 (s, 1H, Ar-*H*), 7.98 (d, *J* = 10.0 Hz, 1H, Ar-

H), 8.06 (s, 1H, Ar-*H*), 8.12 (d, *J* = 10.0 Hz, 1H, Ar-*H*);ES-MS *m/z* 482 (M+, 100), 484 (M+2, 48); Anal.Calcd for $C_{25}H_{24}ClN_3O_3S$: C, 62.30; H, 5.02; N, 8.72; Found: C, 62.21; H, 5.06; N, 8.69.

3-(3-(6-chloro-2-methoxyacridin-9-ylamino)propyl)-2-(thiophen-2-yl)-1,3-thiazinan-4-one (25)

Yellow solid; Yield 70%; mp 128-129 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.83-1.87 (m, 2H, CH_2), 2.78-2.82 (m, 2H, CH_2), 2.89-2.92 (m, 2H, CH_2), 3.10-3.14 (m, 2H, CH_2), 3.86-3.90 (m, 2H, CH_2), 3.99 (s, 3H, OCH_3), 5.47 (s, 1H, SC*H*N), 6.27 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 6.36 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 6.40 (br s, 1H, N*H* D_2O-exchangeable), 7.27 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.28 (s, 1H, Ar-*H*), 7.38-7.41 (m, 2H, Ar-*H*), 7.54 (s, 1H, Ar-*H*), 7.96 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.04 (s, 1H, Ar-*H*), 8.10 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 498 (M+, 100), 500 (M+2, 58); Anal.Calcd for $C_{25}H_{24}ClN_3O_2S_2$: C, 60.29; H, 4.86; N, 8.44; C, 62.30; H, 5.02; N, 8.72; Found: C, 60.32; H, 4.75; N, 8.42.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-pyridin-4-yl-[1,3]thiazinan-4-one (26)

Yellow solid; Yield 65%; mp 154-155 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.82-1.89 (m, 2H, CH_2), 2.71-2.75 (m, 2H, CH_2), 2.80-2.88 (m, 2H, CH_2), 2.92-2.96 (m, 2H, CH_2), 3.51-3.55 (m, 2H, CH_2), 4.02 (s, 3H, OCH_3), 5.40 (s, 1H, SC*H*N), 6.28 (br s, 1H, N*H* D_2O-exchangeable), 7.17 (d, *J* = 10.0 Hz, 2H, Ar-*H*), 7.28 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.41 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.72 (s, 1H, Ar-*H*), 7.98 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 8.06 (s, 1H, Ar-*H*), 8.10 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.06 (s, 1H, Ar-*H*), 8.10 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.66 (d, *J* = 10.0 Hz, 2H, Ar-*H*); ES-MS *m/z* 494 (M+, 100), 496 (M+2, 38); Anal.Calcd for $C_{26}H_{25}ClN_4O_2S$: C, 63.34; H, 5.11; N, 11.36; Found: C, 63.28; H, 5.17; N, 11.29.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-quinolin-4-yl-[1,3]thiazinan-4-one (27) Yellow solid; Yield 62%; mp 161-163 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.42-1.46 (m,

2H, CH_2), 2.75-2.78 (m, 4H, CH_2), 3.04-3.07 (m, 2H, CH_2), 3.59-3.64 (m, 2H, CH_2), 4.03 (s, 3H, OCH_3), 6.17 (s, 1H, SC*H*N), 6.46 (br s, 1H, N*H* D_2O-exchangeable), 7.11 (d, *J* = 5.0 Hz, 2H, Ar-*H*), 7.40 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.65 (s, 1H, Ar-*H*), 7.67 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.75 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.78 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.81 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.85 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.98 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.06 (s, 1H, Ar-*H*), 8.11 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 8.21 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 544 (M+, 100), 546 (M+2, 58); Anal.Calcd for $C_{30}H_{27}ClN_4O_2S$: C, 66.35; H, 5.01; N, 10.32; Found: C, 66.39; H, 4.98; N, 10.27.

3-[3-(6-Chloro-2-methoxy-acridin-9-ylamino)-propyl]-2-cyclohexyl-[1,3]thiazinan-4-one (28)

Orange yellow solid; Yield 60%; mp 171-173 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.42-1.56 (m, 6H, CH_2-cyclohexyl), 1.72-1.77 (m, 4H, CH_2-cyclohexyl), 1.83-1.87 (m, 2H, CH_2), 2.82-2.85 (m, 1H, CH-cyclohexyl), 2.93-3.01 (m, 4H, CH_2), 3.21-3.25 (m, 2H, CH_2), 4.04 (s, 3H, OCH_3), 4.52 (s, 1H, SC*H*N), 6.34 (br s, 1H, N*H* D_2O-exchangeable), 7.30-7.32 (d, *J* = 10.0 Hz, 1H, Ar-*H*), 7.42 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 7.59 (s, 1H, Ar-*H*), 8.00 (d, *J* = 5.0 Hz, 1H, Ar-*H*), 8.08 (s, 1H, Ar-*H*), 8.17 (d, *J* = 10.0 Hz, 1H, Ar-*H*); ES-MS *m/z* 498 (M+, 100), 500 (M+2, 52); Anal.Calcd for $C_{27}H_{32}ClN_3O_2S$: C, 65.11; H, 6.48; N, 8.44; Found: C, 65.19; H, 6.41; N, 8.49.

Biological assays

All of the cell lines used were purchased from American Tissue Culture Collection (ATCC) (Manassas, VA) and cultured according to supplier's instructions, unless stated otherwise. Cell line authentication was carried out by Genetica DNA Laboratories (Burlington, NC) using a short tandem repeat (STR) profiling method (March 2015; July 2015; September 2016)

Reagents

Chloroquine diphosphate (CQ), quinacrine dihydrochloride (QC), and cisplatin (CP) were purchased from Sigma-Aldrich Canada Ltd (Oakaville, ON, Canada). All the compounds used in the experiments were dissolved in 10-20 mM dimethyl sulfoxide (DMSO) and stored at -20 °C until use. The stock solution was diluted in culture medium (0.1–100 µM) immediately prior to

use. The final concentration of DMSO in the SRB-based cytotoxicity assays did not exceed 0.1%. To rule out that the DMSO concentration used may affect cell proliferation, culture medium containing equivalent concentration of DMSO was used as a negative control in all experiments. In all studies, the concentration of DMSO used did not notably show any anti-proliferative effect.

SRB assay

Anti-proliferative/anti-growth effects were determined by a Sulphorhodamine B (SRB)-based protocol.[8,23] For a typical screening experiment, 5,000-10,000 cells were inoculated into 100 µl medium per well of a 96-well microtiter plate as described previously. Briefly, after the inoculation, the microtiter plate was incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h, prior to addition of experimental drugs. Some of the sample wells were fixed with 25 µl of 50% tricholoroacetic acid (TCA) as a control of the cell population for each cell line at the time of adding a drug (Tz). An aliquot of the frozen stock was thawed and diluted to the desired final maximum test-concentration with complete medium. Two- to ten-fold serial dilutions were made to provide a total of seven drug concentrations (and a control [C]). Following addition of drug(s), the culture plate was incubated for additional 48 h. Cells were fixed *in situ* by slowly adding 25 µl of ice-cold 50% (w/v) TCA (final concentration, 10% TCA), and were then incubated for 60 min at 4 °C. The supernatant was discarded, and the plate was washed five times with tap water, followed by air-dry. 50 µl of SRB solution at 0.4% (w/v) in 1% acetic acid was added to each well, and the plate was incubated for > 30 min at room temperature. Unbound SRB was removed by five washes with tap water, followed by air-drying. The cells "stained" with SRB were solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515-564 nm. The relative growth rate (%) was calculated for each of the compound concentrations according to the following formula:

$$(Ti - Tz)/(C - Tz) \times 100$$

Where Tz, C and Ti denote *time zero*, *control growth* and *OD* for different concentration of tested compounds, respectively. The IC_{50} for each compound was obtained from a non-linear sigmoidal dose-response (variable slope) curve which is fitted by GraphPad Prism v.4.03

software. Values were calculated for each of these parameters if the level of activity was reached. However, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested [8, 31] .

Western blot assay

Samples treated with compound **25** and sham control were collected at scheduled time points post-treatment and centrifuged for 5 min at 1100 rpm (AllegraTM X-12 centrifuge, Beckman Coulter). Cell pellets were washed with PBS by centrifugation for 5 min at 1100 rpm (AllegraTM X-12 centrifuge), and were then lysed with 100 µl Lysis buffer (150 mmol NaCl, 5 mmol EDTA, 1% Triton X-100, 10 mmol Tris pH 7.4, 1 mmol PMSF, 5 mmol EDTA and 5 mmol protease inhibitors cocktail) by maintaining on ice for 10–15 min, followed by centrifugation at 1100 rpm (AllegraTM X- 12 centrifuge) for 10 min at 4 ºC. Supernatant was collected and protein concentration was determined using a BCA protein assay kit (Thermo Scientific, USA). Cell lysates were diluted with 2 X Laemmli sample buffer, and then boiled at 95–100 °C for 5 min. 30–40 µg protein was resolved by polyacrylamide gel (8% or 10%) electrophoresis. The resolved proteins were transferred to a PVDF membrane using a semi-dry gel transfer apparatus (75 min at 24 V), followed by 'blocking' with 5% skim milk for 1 h. Proteins on the membrane were then incubated with primary antibody in 0.1% TBST buffer containing 5% skim milk for overnight at 4 C. Membrane was washed three times with 0.1% TBST buffer and incubated with secondary antibody in TBST buffer containing 5% skim milk for 1 h. Finally, the membrane was washed with TBST buffer thrice, and signals were visualized on X-ray film using an ECL chemiluminescence kit (Super Signal West pico, Thermo Scientific, USA). Antibodies used were purchased from Abcam (Canada) or Santa Cruz biotechnology (Canada) [32].

Clonogenic assay

Cancer cell lines were seeded in 6-well plates at a density of 1000 cells per well. After overnight, test compounds were added and incubated for 12 h. Then the medium was changed and the cells were cultivated until cells in control plates formed sufficiently large colonies. Colonies were then stained with crystal violet staining solution [32].

Acknowledgements

HL wishes to thank to funders: the Natural Sciences and Engineering Council of Canada (NSERC), Northern Cancer Research Foundation, Northern Ontario Heritage Fund Corporation and the Greater Sudbury Business Development Corporation. VRS wishes to acknowledge the Ontario Ministry of Research and Innovation for postdoctoral fellowship.

References

[1] K.D. Miller, R.L. Siegel, C.C. Lin, A.B. Mariotto, J.L. Kramer, J.H. Rowland, K.D. Stein, R. Alteri, A. Jemal, Cancer treatment and survivorship statistics, 2016, CA Cancer J. Clin. 66 (2016) 271-289.
[2] T. Shien, T. Kinoshita, C. Shimizu, T. Hojo, N. Taira, H. Doihara, S. Akashi-Tanaka, Primary tumor resection improves the survival of younger patients with metastatic breast cancer, Oncol. Rep. 21 (2009) 827-832.
[3] F. Rojo, J. Albanell, A. Rovira, J.M. Corominas, F. Manzarbeitia, Targeted therapies in breast cancer, Semin. Diagn. Pathol. 25 (2008) 245-261.
[4] A. Thomsen, J.M. Kolesar, Chemoprevention of breast cancer, Am. J. Health Syst. Pharm. 65 (2008) 2221-2228.
[5] V.R. Solomon, C. Hu, H. Lee, Design and synthesis of anti-breast cancer agents from 4-piperazinylquinoline: a hybrid pharmacophore approach, Bioorg. Med. Chem. 18 (2010) 1563-1572.
[6] V.R. Solomon, C. Hu, H. Lee, Hybrid pharmacophore design and synthesis of isatin-benzothiazole analogs for their anti-breast cancer activity, Bioorg. Med. Chem. 17 (2009) 7585-7592.
[7] B. Meunier, Hybrid molecules with a dual mode of action: dream or reality?, Acc. Chem. Res. 41 (2008) 69-77.
[8] V.R. Solomon, D. Almnayan, H. Lee, Design, synthesis and characterization of novel quinacrine analogs that preferentially kill cancer over non-cancer cells through the downregulation of Bcl-2 and up-regulation of Bax and Bad. Eur. J. Med. Chem.137 (2017)156-166.
[9] S.K. Bhanot, M. Singh, N.R. Chatterjee, The chemical and biological aspects of fluoroquinolones: reality and dreams, Curr. Pharm. Des. 7 (2001) 311-335.
[10] J. Adamec, R. Beckert, D. Weiss, V. Klimesova, K. Waisser, U. Mollmann, J. Kaustova, V. Buchta, Hybrid molecules of estrone: new compounds with potential antibacterial, antifungal, and antiproliferative activities, Bioorg. Med. Chem. 15 (2007) 2898-2906.
[11] V.V. Kouznetsov, A. Gomez-Barrio, Recent developments in the design and synthesis of hybrid molecules based on aminoquinoline ring and their antiplasmodial evaluation, Eur. J. Med. Chem. 44 (2009) 3091-3113.
[12] A. Wozniacka, A. Carter, D.P. McCauliffe, Antimalarials in cutaneous lupus erythematosus: mechanisms of therapeutic benefit, Lupus. 11 (2002) 71-81.
[13] T.B. Gardner, D.R. Hill, Treatment of giardiasis. Clin. Microbiol. Rev. 14 (2001) 114-28.

[14] R. Preet, P. Mohapatra, S. Mohanty, S.K. Sahu, T. Choudhuri, M.D. Wyatt, C.N. Kundu, Quinacrine has anticancer activity in breast cancer cells through inhibition of topoisomerase activity, Int. J. Cancer. 130 (2012) 1660-1670.
[15] P. Mohapatra, R. Preet, D. Das, S.R. Satapathy, T. Choudhuri, M.D. Wyatt, C.N. Kundu, Quinacrine-mediated autophagy and apoptosis in colon cancer cells is through a p53- and p21-dependent mechanism, Oncol. Res. 20 (2012) 81-91.
[16] R. Preet, P. Mohapatra, D. Das, S.R. Satapathy, T. Choudhuri, M.D. Wyatt, C.N. Kundu, Lycopene synergistically enhances quinacrine action to inhibit Wnt-TCF signaling in breast cancer cells through APC, Carcinogenesis. 34 (2013) 277-286.
[17] S.R. Satapathy, S. Siddharth, D. Das, A. Nayak, C.N. Kundu, Enhancement of Cytotoxicity and Inhibition of Angiogenesis in Oral Cancer Stem Cells by a Hybrid Nanoparticle of Bioactive Quinacrine and Silver: Implication of Base Excision Repair Cascade, Mol. Pharm. 12 (2015) 4011-4025.
[18] A. Gomes, I. Fernandes, C. Teixeira, N. Mateus, M.J. Sottomayor, P. Gomes, A Quinacrine Analogue Selective Against Gastric Cancer Cells: Insight from Biochemical and Biophysical Studies, Chem.Med. Chem. (2016).
[19] A. Geronikaki, P. Eleftheriou, P. Vicini, I. Alam, A. Dixit, A.K. Saxena, 2-Thiazolylimino/heteroarylimino-5-arylidene-4-thiazolidinones as new agents with SHP-2 inhibitory action, J. Med. Chem. 51 (2008) 5221-5228.
[20] N.S. Cutshall, C. O'Day, M. Prezhdo, Rhodanine derivatives as inhibitors of JSP-1, Bioorg. Med. Chem. Lett. 15 (2005) 3374-3379.
[21] A. Degterev, A. Lugovskoy, M. Cardone, B. Mulley, G. Wagner, T. Mitchison, J. Yuan, Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-xL, Nat. Cell Biol. 3 (2001) 173-182.
[22] V. R. Solomon, C. Hu, H. Lee, Design and synthesis of chloroquine analogs with anti-breast cancer property, Eur. J. Med. Chem. 45 (2010) 3916-3923.
[23] V.R. Solomon, W. Haq, K. Srivastava, S.K. Puri, S.B. Katti, Synthesis and antimalarial activity of side chain modified 4-aminoquinoline derivatives, J. Med. Chem. 50 (2007) 394-398.
[24] J. Knockleby, H. Lee, Same partners, different dance: involvement of DNA replication proteins in centrosome regulation, Cell Cycle. 9 (2010) 4487-4491.
[25] A.S. Hemerly, S.G. Prasanth, K. Siddiqui, B. Stillman, Orc1 controls centriole and centrosome copy number in human cells, Science. 323 (2009) 789-793.
[26] J. Gautier, J.L. Maller, Cyclin B in Xenopus oocytes: implications for the mechanism of pre-MPF activation, EMBO J. 10 (1991) 177-182.
[27] J. Bartek, J. Lukas, Chk1 and Chk2 kinases in checkpoint control and cancer, Cancer Cell. 3 (2003) 421-429.
[28] P.L. de Souza, M. Castillo, C.E. Myers, Enhancement of paclitaxel activity against hormone-refractory prostate cancer cells in vitro and in vivo by quinacrine, Br. J. Cancer. 75 (1997) 1593-1600.
[29] S.A. Santi, H. Lee, Ablation of Akt2 induces autophagy through cell cycle arrest, the downregulation of p70S6K, and the deregulation of mitochondria in MDA-MB231 cells, PLoS One. 6 (2011) e14614.
[30] L.J. Simons, B.W. Caprathe, M. Callahan, J.M. Graham, T. Kimura, Y. Lai, H. LeVine, 3rd, W. Lipinski, A.T. Sakkab, Y. Tasaki, L.C. Walker, T. Yasunaga, Y. Ye, N. Zhuang, C.E. Augelli-Szafran, The synthesis and structure-activity relationship of substituted N-phenyl

anthranilic acid analogs as amyloid aggregation inhibitors, Bioorg. Med. Chem. Lett. 19 (2009) 654-657.
[31] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, New colorimetric cytotoxicity assay for anticancer-drug screening, J. Natl. Cancer Inst. 82 (1990) 1107-1112.
[32] S. Pundir, H.Y. Vu, V.R. Solomon, R. McClure, H. Lee, VR23: A Quinoline-Sulfonyl Hybrid Proteasome Inhibitor That Selectively Kills Cancer via Cyclin E-Mediated Centrosome Amplification, Cancer Res. 75 (2015) 4164-75.

Table 1. Anti-proliferative activity of quinacrine analogs (**6-28**) against human breast cancer and non-cancer breast cell lines

Lab Code	Compound[a]	-R	IC_{50}[b,c]			
			MDA-MB 468	MDA-MB231	MCF7	184B5
VR126	6	phenyl	4.72±0.39	4.07±0.02	4.79±0.34	9.32±0.25
VR139	7	2-*p*-tolyl	6.97±0.52	4.87±0.32	5.52±0.23	5.66±0.21
VR127	8	2-fluoro-phenyl	2.68±0.11	4.11±0.22	2.29±0.11	35.58±0.87
VR128	9	4-fluoro-phenyl	4.58±0.52	5.36±0.31	4.97±0.32	28.23±0.58
VR130	10	2,4-difluoro-phenyl	3.79±0.26	4.54±0.37	3.39±0.45	5.08±0.31
VR131	11	2,6-difluoro-phenyl	3.20±0.11	4.41±0.33	3.81±0.21	29.27±0.24
VR132	12	2-chloro-phenyl	19.92±0.34	10.78±0.54	15.35±0.54	69.56±0.58
VR133	13	4-chloro-phenyl	4.43±0.55	3.53±0.23	3.40±0.32	5.02±0.12
VR134	14	2,4-dichloro-phenyl	3.72±0.31	3.07±0.31	1.85±0.01	4.28±0.21
VR135	15	2,6-dichloro-phenyl	1.29±0.07	7.54±0.72	4.41±0.23	3.84±0.21
VR136	16	2-chloro-6-fluoro phenyl	5.19±0.41	3.44±0.56	4.31±0.22	3.1±0.11
VR137	17	2-bromo-phenyl	38.58±0.34	40.08±1.03	39.33±0.65	15.6±0.28
VR138	18	4-bromo-phenyl	25.53±0.65	37.10±0.98	31.31±0.35	35.81±0.65
VR140	19	4-dimethylamino-phenyl	3.56±0.41	2.63±0.02	3.09±0.21	13.19±0.28
VR141	20	4-diphenylamino-phenyl	1.68±0.38	3.32±0.13	1.85±0.23	3.11±0.21
VR142	21	2-nitro-phenyl	1.21±0.06	0.77±0.11	0.99±0.11	1.83±0.11
VR143	22	4-nitro-phenyl	9.41±0.88	4.68±0.25	7.04±0.21	19.51±0.25
VR150	23	2-1*H*-pyrrol-2-yl	4.87±0.05	4.56±0.33	1.66±0.11	12.86±0.36
VR146	24	2-furan-2-yl	3.67±0.21	3.46±0.02	3.57±0.21	3.6±0.21
VR151	25	2-thiophen-2-yl	1.73±0.80	2.80±1.30	0.69±0.41	4.96±0. 24
VR147	26	2-pyridin-4-yl	21.58±0.21	10.78±0.15	16.18±0.45	14.62±0.23
VR144	27	2-quinolin-4-yl	10.40±0.18	7.48±0.31	8.94±0.51	27.1±0.64
VR145	28	2-cyclohexyl	5.25±0.21	5.85±0.43	5.05±0.22	7.71±0.24
	CQ		28.58±0.25	22.52±1.44	38.44±0.32	76.13±0.23
	Cisplatin		31.02±0.32	23.63±0.23	25.77±0.65	25.54±0.56
	Quinacrine		3.96±0.12	3.25±0.11	4.19±0.14	4.96±0.16

[a] Chemical structures are shown in Scheme 1. [b] IC_{50} values were calculated from sigmoidal dose response curves (variable slope), which were generated with GraphPad Prism V. 4.02 (GraphPad Software Inc.). [c] Values are the mean of triplicates of at least two independent experiments. CQ-Chloroquine diphosphate

Table 2: Summary of cell viability against compound **25** (VR151)

Cell lines	Cell characteristics	VR151 (µM)*
MCF7	Mammary adenocarcinoma, ER+	1
BT20	Mammary carcinoma, ER-, IRS-1-/-	1
BT474	Mammary carcinoma, Her2++, p53-	1
SkBr3	Mammary carcinoma, Her2++, ER-	2
CAMA1	Mammary carcinoma, PTEN-, ER+,Her2+, c-Myc++	2
MDA-MB453	Mammary adenocarcinoma, PTEN-	2
MDA-MB231	Mammary carcinoma, ER-, PR_, p53-/-, K-ras mutant	2
MDA-MB468	Mammary carcinoma, PTEN-/-, RB1-/-, p53-/-, ER-	2
LNCap	Prostate cancer, JAK-1 mutant, androgen dependent,	2
PC-3	Prostate cancer, EGFR mutant, androgen independent	2
A549	Non-small cell lung adenocarcinoma, K-ras mutant	2
NCI-H1975	Non-small cell lung cancer, EGFR-, p53-	2
MCF10A**	Non-malignant mammary epithelial cell	>6
184B5**	Non-malignant mammary epithelial cell	6

* At these VR151 concentrations, cell viability measured by counting number of viable cells was nearly completely inhibited. **Non-malignant cells were not inhibited at 1-2 µM of VR151; however, they were inhibited at 6 µM or higher concentrations.

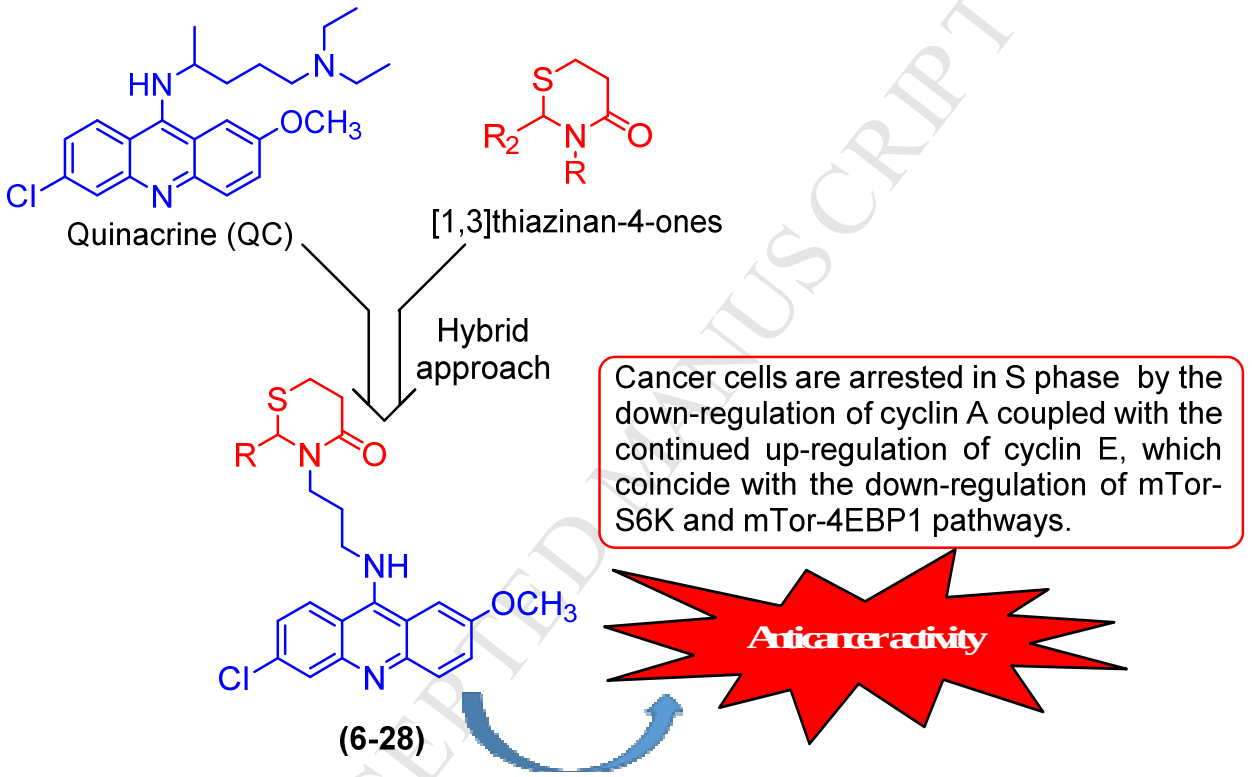


Fig. 1. The design of hybrid compounds from quinacrine and [1,3]thiazinan-4-ones.

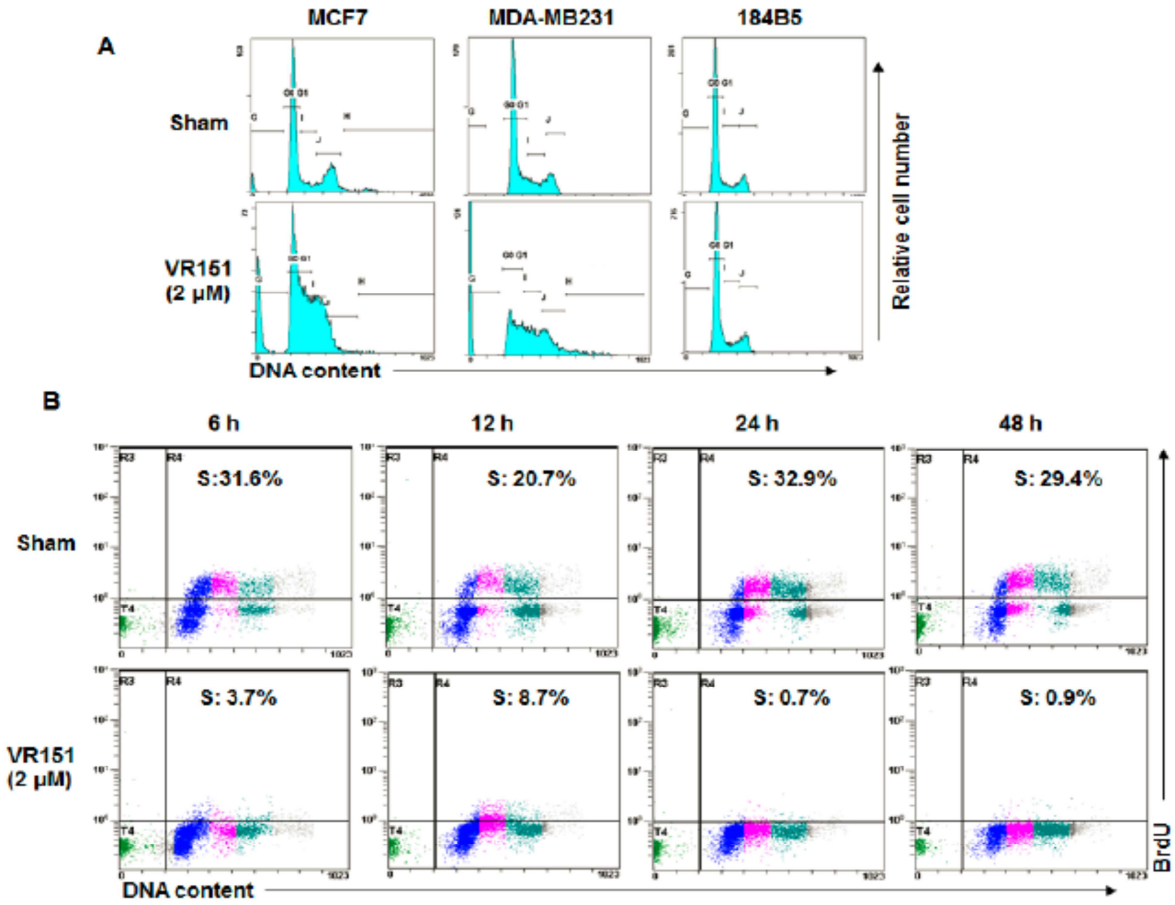


Figure 2. Compound **25** (VR151) arrested cell cycle at S phase in a cancer cell-specific manner. (A) MCF7 and MDA-MB231 cancer cells and matching 184B5 non-cancer breast cells were exposed to 2 μM of compound **25** for 48 h, followed by cell cycle analysis by flow cytometry. Experiment was carried out in duplicates, which was repeated three times. (B) MCF7 cells were sham-treated or treated with 2 μM of compound **25** for 6, 12, 24 or 48 h. Cells were exposed to 10 μM BrdU for 45 min immediately prior to the termination of the experiment, followed by immunostaining with an anti-BrdU antibody. DNA was counterstained with propidium iodide (PI) (X-axis). Representative plots are shown from four independent trials.

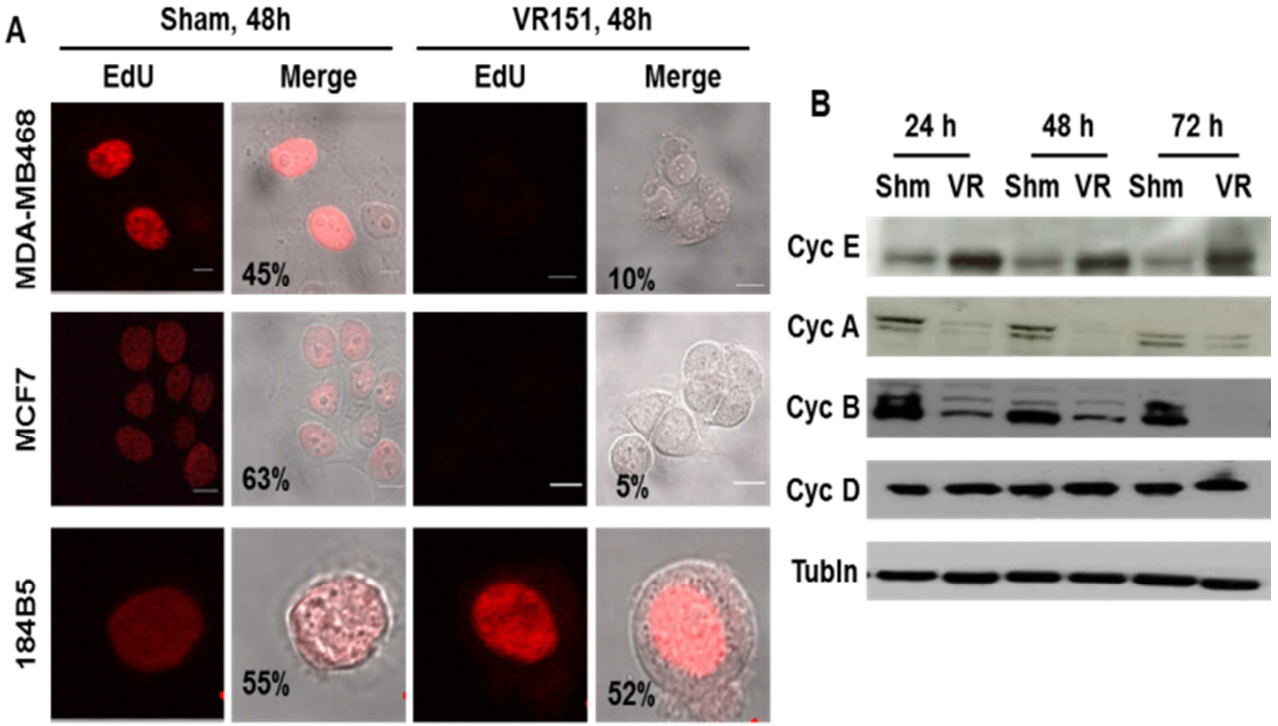


Figure 3. Cancer cells do not replicate DNA while maintaining a high level of cyclin E and low levels of cyclins A and B in the presence of compound **25**. (A) Cancer cells do not replicate DNA. MDA-MB468, MCF7 or 184B5 cells were sham-treated or exposed to 2 μM of compound **25** (VR151) for 48 h, the final 45 min of which was in the presence of 10 μM EdU. Cells with EdU-labeled DNA were counted in 10 random fields. Results shown are exemplary pictures of one trial from two independent experiments performed in duplicates. The number of % within each picture is % of EdU-labeled cells. Bar is 5 μm in length in MCF7 and MDA-MB231 and 2 μm in 184B5. (B) Cells contained high levels of cyclin E ($p = <0.0001$ at 12 and 24h) but low levels of cyclin A ($p\text{-value}<0.0001$) and cyclin B ($p\text{-value}<0.0001$ at 24, 48 and 72h) in the presence of compound **25**. MCF7 cells were sham-treated (Shm) or treated with 2 μM of compound **25** (VR) for 24, 48 or 72 h, followed by protein separation by SDS-PAGE and Western blotting. Cyc and Tubln denote cyclin and tubulin, respectively.

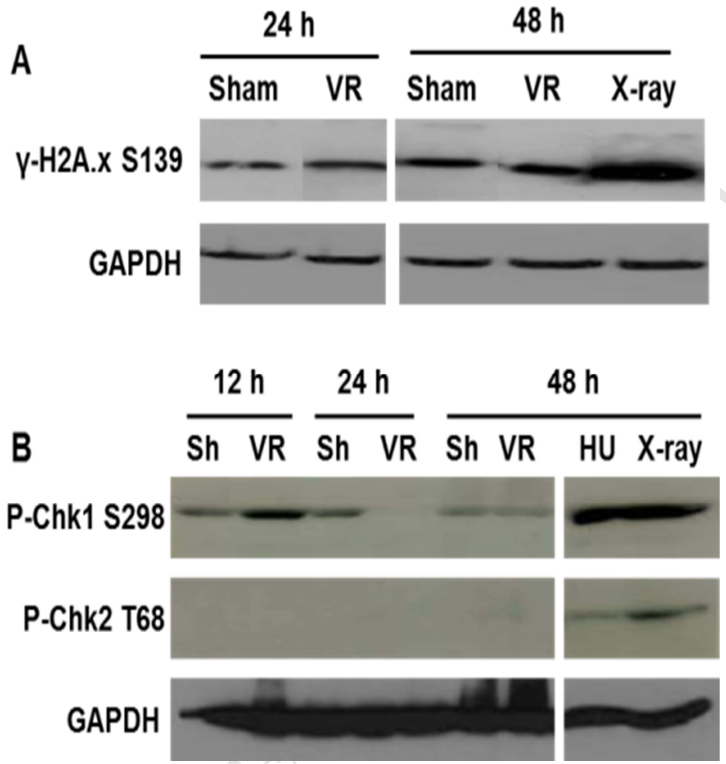


Figure 4. Compound **25** (VR) did not cause DNA damage. (A) γ-H2A.x (Ser139) levels were not increased beyond the sham-control in MCF7 cells treated with compound **25** (2 μM). (B) The levels of Chk1 and Chk2 were not increased in the presence of 2 μM compound **25**. HU denotes hydroxyurea (1 mM). X-ray was 10 Gy at room temperature.

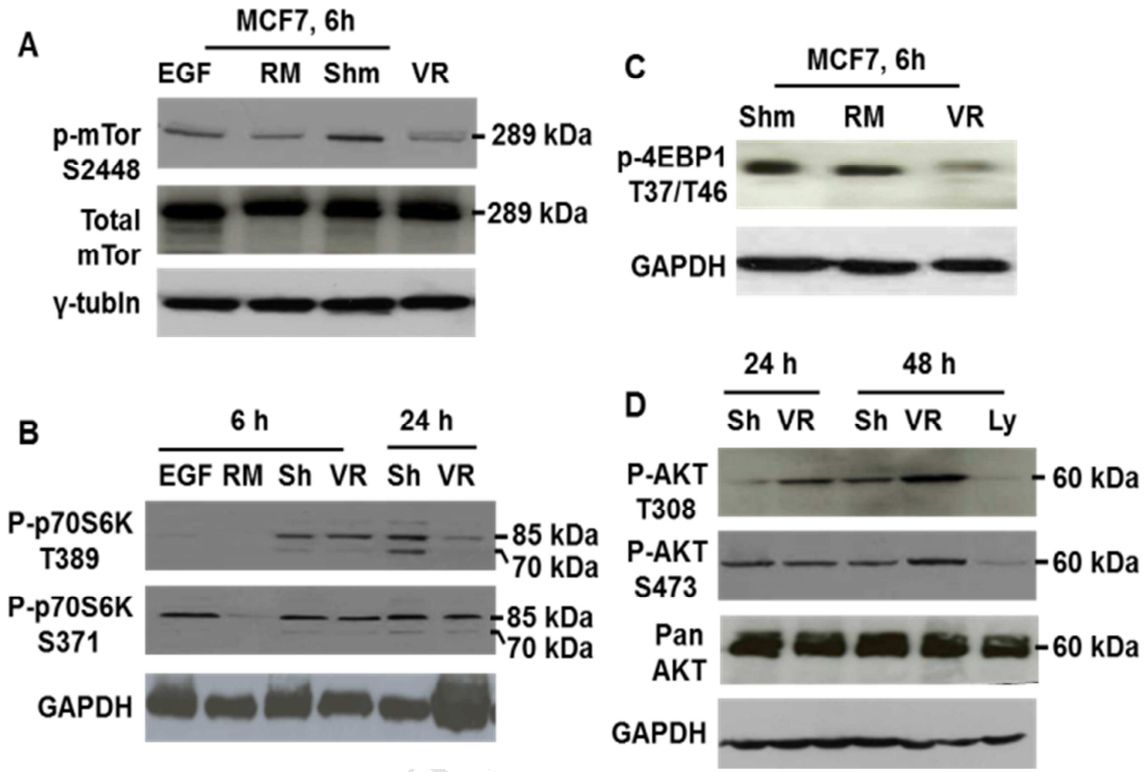
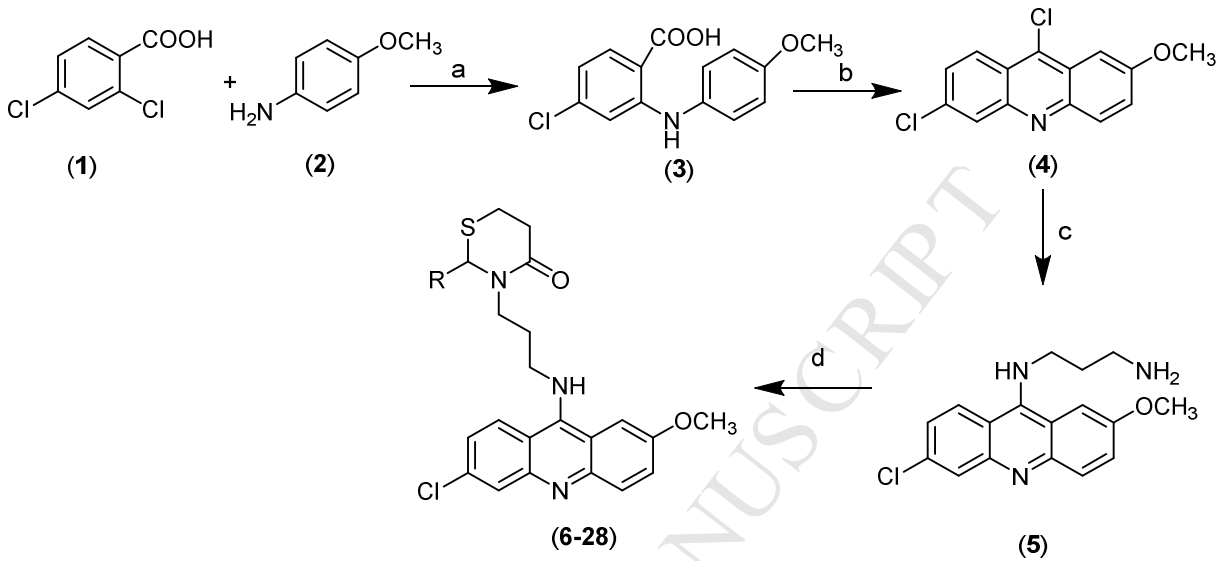


Figure 5. The mTOR-p70/p85S6K-4EBP1 pathway was down-regulated in the presence of compound **25** (VR151). (A) MCF7 cells treated with compound **25** (2 μM) showed a decrease in the phosphorylation level of mTOR on SerS2448 by 6 h post-treatment ($p<0.0001$). EGF, RM, Sh and VR denote epidermal growth factor, rapamycin, sham control and VR151 (compound **25**), respectively. (B) Compound **25** (2 μM) down-regulated S6K on Thr389 by 24 h post-treatment. (C) The 4EBP1 activity is down-regulated within 6 h in the presence of compound **25** (2 μM). (D) Akt activity is slightly up-regulated in the presence of compound **25**. Ly denotes LY294009. Western blots shown are representative of three independent experiments.



Scheme 1.Synthesis of novel quinacrine analogs. Reagents and conditions*:* (a) $LiNH_2$, THF, 8 h; (b) $POCl_3$, 120-130 ^oC for 3 h; (c) Triethyl amine, 1,3-diamino propane, 120-130 ^oC for 6 h; (d) R-CHO, Mercapto propionic acid, DCC, THF, room temperature, 1h.

Highlights

• A series of novel quinacrine derived thiazinan-4-ones were synthesized.

• The detailed SARs of the derivatives were summarized.

• Compound **25** is substantially effective than the parental quinacrine.

• Compound **25** bind to certain protein essential for DNA replication without causing DNA damage, resulting in the inhibition of DNA replication and/or the down-regulation of cyclin A.