Synthesis, computational study and biological evaluation of 9-acridinyl and 1-coumarinyl-1,2,3-triazole-4-yl derivatives as topoisomerase II inhibitors

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INTRODUCTION

Second to cardiovascular diseases, cancer is the most common cause of death. The urgent need to combat cancer, necessitates developing more effective anticancer agents. Molecular hybridisation in drug design is based on the combination of pharmacophoric moieties of bioactive molecules to produce a new hybrid compound with better activity in comparison with the parent compounds. Practice of this technique has afforded bioactive compounds with improved efficacy relative to the component individual subunits.

The ability of the tricyclic planar aromatic structure of acridine to intercalate in the double-stranded DNA structure and the acridine nitrogen adoption of an acceptor or donor conformation, which has a radical effect on the binding properties of the molecule are the major considerations for the use of acridines as anticancer compounds. In addition, acridine derivatives prevent abnormal functioning of tumour cells, which includes dysfunction of the enzymes that control the topology of DNA. Topoisomerase, telomerase, and cyclin-dependent kinases (CDKs) are illustrative examples of enzymes inhibited by acridine derivatives such as 9-azidoacridine/3-azidocoumarin with N/O-propargyl small molecules under click reaction conditions. Cancer cell growth inhibition of the synthesised triazoles was tested against human cell-lines in the NCI-60 cell-panel, and the most active compounds tested against topoisomerase (II)-enzymes. The acridinyl ligands revealed 60–97% cell growth inhibition in six cancer cell panels. Cell-cycle analysis of MCF7 and DU-145 cells treated with the active acridinyl ligands exhibited cell-cycle arrest at G2/M phase and proapoptotic activity. In addition, compound displayed greater inhibitory activity against topoisomerase (II) (IC50 0.52 μM) compared with doxorubicin (IC50 0.83 μM). Molecular dynamics simulation studies showed the acridine-triazole-pyrimidine hybrid pharmacophore was optimal with respect to protein-ligand interaction and fit within the binding site, with optimal orientation to allow for intercalation with the DNA bases (DG13, DC14, and DT).
and several kinases, including PI3K, have been implicated in the anticancer mechanism of action resulting in the induction of cell-cycle arrest and apoptosis\(^23,24\).

The triazole core mimics an amidic bond and is planar with hydrogen bond donor and acceptor properties; however, in contrast to the amidic bond triazoles are metabolically stable\(^25\). Moreover, the hydrogen bonding properties of the triazole ring enable formation of C-H...π interactions with the respective enzyme or receptor target\(^26\). The physicochemical properties and pharmacological potential of the 1,4-disubstituted triazole ring combined with the conformational flexibility of the designed ligands are favourable properties in the design of novel bioactive compounds.

### Experimental

#### Chemistry

All the chemicals used for the synthesis of the target compounds were of commercially available analytical grade. TLC was carried out using silica gel 60 F\(_{254}\) pre-coated sheets 20 x 20 cm, layer thickness 0.2 mm (E. Merck, Darmstadt, Germany), and spots were visualised using UV-lamp at \(λ_{max}\) 254 nm. Column chromatography was performed using Fluka silica gel 60 (particle size 0.063–0.02 mm). Melting points were measured using an electrothermal apparatus (Stuart Scientific, Redhill, UK), and were uncorrected. Infra-red spectra were determined as KBr disks using a Thermo Scientific Nicolet 6700 FT-IR spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at the Pharmaceutical Service Laboratory, Faculty of Pharmacy, Assiut University, Assiut, Egypt. \(^1\)H NMR and \(^13\)C NMR spectra were performed on a Bruker Spectrophotometer (Billerica, MA) operating at 400 MHz and 100 MHz, respectively. Chemical shifts are expressed in \(δ\) values (ppm) relative to tetramethylsilane (TMS) as an internal standard and CDCl\(_3\) and DMSO-d\(_6\) were used as solvents. D\(_2\)O was used for the detection of exchangeable protons. Elemental microanalyses were conducted at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. 6-Methyluracil and propargyl bromide (80% w/v in toluene) were obtained from Sigma Aldrich Co. (Darmstadt, Germany). Preparation and analytical data of compounds 1–2 were as reported\(^27–31\) and 3–7 also as reported\(^32–35\).

**General synthesis of 1,4-disubstituted-1,2,3-triazoles** – In a mixture of water and t-butanol (10 ml, 1:1 v/v), the respective azides (1–2) (1 mmol) and propargyl derivatives (3–7) (1 mmol) were suspended. Sodium ascorbate (0.1 mmol) was added then a solution of copper (II) sulphate pentahydrate (0.01 mmol, 100 \(μL\) water). The mixture was heated at 80°C overnight. The precipitate produced was collected by vacuum filtration, washed with water, dried at 50°C and purified by column chromatography (EtOAc:hexane, 2:3 v/v).

1-{(1-(Acridin-9-yl)-1H-1,2,3-triazol-4-yl)methyl}-6-methylpyrimidine-2,4(1H,3H)-dione (8)

White solid, yield: 57% (200 mg); mp: 247–250°C. FT-IR (KBr, cm\(^{-1}\)): 3181, 3162, 3005, 1715, and 1682. \(^1\)H NMR (DMSO-d\(_6\) \(δ\)): 11.3 (1H, s, NH, exchangeable), 8.86 (1H, s, H5-triazole), 8.34 (2H, d, \(J = 8.7\) Hz, H4, H5-acridine), 7.98 (2H, t, \(J = 7.6\) Hz, H2, H7-acridine), 7.74 (2H, t, \(J = 7.6\) Hz, H2, H7-acridine), 7.37 (2H, d, \(J = 8.7\) Hz, H1, H8-acridine), 5.62 (1H, s, CH-methyluracil), 5.29 (2H, s, CH2), 3.33 (3H, s, CH3). \(^13\)C NMR (DMSO-d\(_6\) \(δ\)): 163.05, 154.72, 152.06, 149.12, 134.41, 131.17, 130.96, 127.98, 126.87, 124.56, 120.58, 118.22, 118.06, 110.53, 54.52, 40.59, 25.58, 18.91.

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Figure 1. Examples of acridine compounds with anticancer activity.

Figure 2. Designed acridine-triazole-drug and coumarin-triazole-drug hybrid compounds.
1-((Acridin-9-yl)-1H,1,2-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione (9)

White solid, yield: 61% (200 mg); mp: 254–256 °C. FT-IR (KBr, cm⁻¹): 3183, 3155, 3049, 1705, 1682, and 1630. ¹H NMR (DMSO-d₆): δ: 11.38 (1H, s, NH, exchangeable), 8.87 (1H, s, H₅-triazole), 8.34 (2H, d, J = 8.7 Hz, H₄, H₅-acridine), 7.98 (2H, t, J = 7.6 Hz, H₃, H₆-acridine), 7.94 (1H, d, J = 7.9 Hz, H₆-uracil), 7.72 (2H, t, J = 7.6 Hz, H₂, H₇-acridine), 7.38 (2H, d, J = 8.7 Hz, H₁, H₈-acridine), 5.68 (1H, d, J = 7.8 Hz, H₅-uracil), and 5.22 (2H, s, CH₃). ¹³C NMR (DMSO-d₆): δ: 164.26, 151.38, 149.12, 146.13, 143.73, 137.82, 131.71, 129.83, 129.03, 128.40, 122.75, 122.06, 101.95, and 49.26. Elemental analyses, found: C%, 65.02; H%, 3.85; N%, 22.93. Calc for C₃₀H₂₈N₁₀O₂ (MW 530.57): C%, 64.86; H%, 3.81; N%, 22.69.

3-((Acridin-9-yl)-1H,1,2-triazol-4-yl)-5,5-diphenylimidazolidin-2,4-dione (10)

Yellow solid, yield: 80% (370 mg); mp: 263–265 °C. FT-IR (KBr, cm⁻¹): 3213, 3160, 3108, 1700, 1708, and 1628. ¹H NMR (DMSO-d₆): δ: 9.79 (1H, s, NH, exchangeable), 8.84 (1H, s, H₅-triazole), 8.33 (2H, d, J = 8.7 Hz, H₄, H₅-acridine), 7.97 (2H, t, J = 7.6 Hz, H₃, H₆-acridine), 7.68 (2H, t, J = 7.6 Hz, H₂, H₇-acridine), 7.38 (10H, m, H-phenyl), 7.29 (2H, d, J = 8.7 Hz, H₁, H₈-acridine), and 5.00 (2H, s, CH₂). ¹³C NMR (DMSO-d₆): δ: 173.4, 155.33, 149.11, 143.02, 140.03, 137.82, 131.68, 129.84, 129.04, 128.97, 128.72, 128.37, 127.26, 125.56, 122.10, 69.84, and 40.63. Elemental analyses, found: C%, 73.12; H%, 4.41; N%, 16.70. Calc for C₃₇H₂₇N₁₄O₂ (MW 510.55): C%, 72.93; H%, 4.34; N%, 16.46.

6-Methyl-1-((2-oxo-2H-chromen-3-yl)-1H,1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione (11)

White solid, yield: 74% (250 mg); mp: 279–283 °C. FT-IR (KBr, cm⁻¹): 3182 (NH), 3165, 3025, 2784, 1728, 1714, and 1697. ¹H NMR (DMSO-d₆): δ: 11.3 (1H, s, NH exchangeable), 8.71 (1H, s, H-triazole), 8.57 (1H, s, C=CH), 7.93 (1H, d, J = 7 Hz, H₈), 7.76–7.71 (1H, dd, J = 7.82 Hz, 7.8 Hz, 1.12 Hz, H₇), 7.55 (1H, t, J = 8.3 Hz, H₆), 7.49–7.45 (1H, t, J = 7.5 Hz, H₅), 5.56 (1H, s, H-uracil), 5.14 (2H, s, CH₂), and 2.40 (3H, s, CH₃). ¹³C NMR (DMSO-d₆): δ: 163, 156.35, 154.63, 152.91, 151.98, 144.33, 135.59, 133.46, 129.99, 125.82, 125.06, 126.41, 118.57, 117.65, 101.75, 40.52, and 19.93. Elemental analyses, found: C%, 58.34; H%, 3.70; N%, 20.12. Calc for C₁₇H₁₃N₂O₄ (MW 351.32): C%, 58.12; H%, 3.73; N%, 19.93.

1-((2-Oxo-2H-chromen-3-yl)-1H,1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione (14)

White solid, yield: 62% (200 mg); mp: 262–264 °C. FT-IR (KBr, cm⁻¹): 3282, 3185, 3045, 1715, 1700, and 1627. ¹H NMR (DMSO-d₆): δ: 11.35 (1H, s, NH, exchangeable), 8.73 (1H, s, H₅-triazole), 8.63 (1H, s, C=CH), 7.95–7.93 (1H, dd, J = 7.7 Hz, 1.08 Hz, H₈), 7.84 (1H, d, J = 7.8 Hz, H₆-uracil), 7.74 (1H, d, J = 7.85 Hz, 7.8 Hz, 1.4 Hz, H₇), 7.55 (1H, d, J = 8.3 Hz, H₆), 7.47 (1H, t, J = 7.5 Hz, H₅), 5.63 (1H, dd, J = 7.8 Hz, 0.8 Hz, H₅-uracil), and 5.07 (2H, s, CH₂). ¹³C NMR (DMSO-d₆): δ: 164.18, 156.33, 152.91, 151.98, 145.99, 143.20, 135.46, 134.44, 130, 125.82, 125.12, 123.61, 118.61, 116.75, 101.83, and 42.75. Elemental analyses, found: C%, 57.16; H%, 3.31; N%, 20.89. Calc for C₁₉H₁₁N₂O₄ (MW 373.29): C%, 56.98; H%, 3.29; N%, 20.76.

1-((2-Oxo-2H-chromen-3-yl)-1H,1,2,3-triazol-4-yl)methyl)pyrimidine-2,4(1H,3H)-dione (15)

White solid, yield: 50% (230 mg); mp: 238–240 °C. FT-IR (KBr, cm⁻¹): 3339, 1760, 1733, 1710, and 1653. ¹H NMR (DMSO-d₆): δ: 9.75 (1H, s, NH, exchangeable), 8.73 (1H, s, H₅-triazole), 8.56 (1H, s, C=CH), 7.93 (1H, d, J = 7.7 Hz, H₈), 7.73 (1H, dd, J = 11.4 Hz, 7.28 Hz, 1 Hz, H₇), 7.55 (1H, d, J = 8.3 Hz, H₆), 7.47 (1H, t, J = 7.5 Hz, H₅), 7.42–7.33 (10H, m, H-phenyl), and 4.84 (2H, s, CH₂). ¹³C NMR (DMSO-d₆): δ: 173.36, 156.33, 155.29, 152.88, 142.77, 139.97, 135.18, 133.37, 129.97, 129.10–128.71, 127.29, 126.92, 125.79, 124.56, 124.09, 123.63, 118.63, 116.73, 69.84, and 51.02. Elemental analyses, found: C%, 68.11; H%, 4.08; N%, 14.86. Calc for C₂₁H₁₃N₂O₄ (MW 477.47): C%, 67.92; H%, 4.01; N%, 14.67.
1-(1-(2-Oxo-2H-chromen-3-yl)-1H-1,2,3-triazol-4-yl)methyl-(S)-2-(6-methoxyxynaphthalen-2-yl)propanoate (16)

White solid, yield: 46% (200 mg); mp: 142–144 °C. FT-IR (KBr, cm⁻¹): 3182, 1730, 1716, and 1609. ¹H NMR (DMSO-d₆): 8.70 (1H, s, H₅-triazole), 8.56 (1H, s, C=CH), 7.93 (1H, dd, J = 7.18 Hz, 0.08 Hz, H₈), 7.74 (4H, m, H₇, 3H-naphth), 7.55 (1H, d, J = 8.3 Hz, H₆), 7.48 (1H, ddd, J = 0.8 Hz, 7.7 Hz, 7.5 Hz, H₅), 7.39 (1H, dd, J = 8.4 Hz, 1.6 Hz, H₂-naphth.), 7.27 (1H, d, J = 2.3 Hz, H₃-naphth), 5.32 (2H, dd, J = 12 Hz, 21 Hz, CH₃), 3.98 (1H, q, J = 7. CH₃), 3.84 (3H, s, OCH₃), and 1.49 (3H, d, J = 7.12 Hz, CH-CH₃). ¹³C NMR (DMSO-d₆) δ: 174.17, 156.67, 156.27, 152.90 (C), 142.66, 133.89, 133.37, 133.79, 133.42, 129.99, 129.60, 128.82, 127.50, 126.99, 126.10, 126.06, 125.80, 125.50, 119.21, 118.61, 116.75, 106.15 (CH), 57.76, 55.61, 44.76 (CH), and 18.97 (CH₃). Elemental analyses, found: C%, 68.78; H%, 6.14; N%, 9.40. Calc for C₂₀H₂₃N₃O₄ (MW 369.41): C%, 65.03; H%, 6.28; N%, 9.23.

DNA-flow cytometry analysis

MCF7 cell lines (1 × 10⁶ cell density) were subjected to 2.70 μM of compound 8, 10.86 μM of 9, 42.71 μM of 10 and (0.002%) DMSO as a control for 24 h. In the same way, DU-145 cell lines (1 × 10⁶ cell density) were subjected to 26 μM of compound 8, 59.34 μM of 9, and 79.27 μM of 10 and (0.002%) DMSO as a control for 24 h. The cells were trypsinised for detachment, ice-cold PBS was used for washing and ice cold 70% EtOH was used for fixation. Afterwards, the cells remain at least 2 h at 4 °C in EtOH then centrifuged. Thereafter, PBS was used for washing of the cells and then Cycle TESTTM plus DNA Reagent Kit (ab139418_Propidium Iodide Flow Cytometry Kit) was used for staining. FACS-Calibar flow cytometer and Cell Quest software (Becton Dickinson Biosciences, San Jose, CA) were used for determination of cell cycle distribution and analysis, respectively.

Topoisomerase IIB inhibitory assay

This assay utilises human topoisomerase 2-beta (TOP2B) ELISA Kit. In brief, compounds 8–10 were evaluated using 10 folds serial concentration 0.01–100 μM in a six-well plate for 24 h incubation at 37 °C and 5% CO₂. After treatment, cell suspension is diluted with PBS (pH 7.2–7.4), until cell concentration reached 10 million/ml. Then, store overnight at −20 °C. After two freeze–thaw cycles to break up the cell membranes, the cell lysates were centrifuged for 5 min at 2–8 °C; collected the supernatant. Cell lysates should be examined immediately or aliquoted and stored at −20 °C. One hundred microlitres of standard and sample per well was added and incubated for 2 h at 37 °C then 100 μL of biotin-conjugated antibody specific for TOP2B is added to each well. Incubate for 1 h at 37 °C, aspirated each well and wash. After washing, 100 μL of avidin conjugated horseradish peroxidase (HRP) is added to each well; incubated for 1 h at 37 °C. After that 90 μL of TMB Substrate is added to each well and incubated for 15–30 min at 37 °C; protected from light. Finally, 50 μL of stop solution is added to each well and gently tapped the plate to ensure thorough mixing. The optical density of each well within 5 min, using a microplate reader was determined.

Annexin V-FITC apoptosis assay

The most active compounds (8, 9, and 10) were assayed against HL-60 (TB) and MOLT-4 cell lines using Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ) according to standard protocol.

Computational studies

Molecular docking and molecular dynamics (MD) simulations were performed as previously described. Briefly, the compounds were docked using the topoisomerase IIB crystal structure in complex with DNA (PDB ID: 4GUO), to generate PDB files of the topoisomerase IIB-ligand complexes, using molecular operating environment (MOE) until a RMSD gradient of 0.01 kcal/mol/Å with the MMFF94 forcefield (ligands) and partial charges were automatically calculated. Docking was performed using the Alpha Triangle placement to determine the poses, refinement of the results was done using the MMFF94 forcefield and rescoring of the refined results using the London ΔG scoring function was applied. Molecular dynamics simulations were run on the topoisomerase IIB-ligand complexes with the PDB files first optimised with

Biological studies

The anticaner activity of the synthesised compounds was studied using high-efficiency biological screening methodology according to the Developmental Therapeutics Program (DTP) (Bethesda, MD), National Cancer Institute (NCI) (Bethesda, MD) and in the confirmatory diagnostic unit, VACSERA (Cairo, Egypt).

Preliminary in vitro anticancer screening

Compounds (8–17) were submitted to the National Cancer Institute “NCI”; all the submitted compounds were selected for preliminary screening. The compounds were tested at a single dose of 10 μM in the full NCI-60 cell panel.

MTT assay for cell viability

The most active compounds (8, 9, and 10) were tested against MCF7 and DU-145 cells lines using MTT assay according to the standard protocol.

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Molecular docking and molecular dynamics (MD) simulations were performed as previously described. Briefly, the compounds were docked using the topoisomerase IIB crystal structure in complex with DNA (PDB ID: 4GUO), to generate PDB files of the topoisomerase IIB-ligand complexes, using molecular operating environment (MOE) until a RMSD gradient of 0.01 kcal/mol/Å with the MMFF94 forcefield (ligands) and partial charges were automatically calculated. Docking was performed using the Alpha Triangle placement to determine the poses, refinement of the results was done using the MMFF94 forcefield and rescoring of the refined results using the London ΔG scoring function was applied. Molecular dynamics simulations were run on the topoisomerase IIB-ligand complexes with the PDB files first optimised with
protein preparation wizard in Maestro by assigning bond orders, adding hydrogen, and correcting incorrect bond types. A default quick relaxation protocol was used to minimise the MD systems with the Desmond programme of Schrodinger. The orthorhombic water box allowed for a 10 Å buffer region between protein atoms and box sides. Overlapping water molecules were deleted, and the systems were neutralised with Na\(^+\) ions and salt concentration of 0.15 M. Force-field parameters for the complexes were assigned using the OPLS 2005 forcefield, that is, a 200 ns molecular dynamic run in the NPT ensemble \((T = 300 \text{ K})\) at a constant pressure of 1 bar. Energy and trajectory atomic coordinate data were recorded at each 1.2 ns and a total of 1000 frames generated. Prime/MMGBSA\(^{45}\), available in Schrodinger Prime suite, was used to calculate the binding free energy of the ligands with topoisomerase IIB in complex with DNA.

### Table 1. Yields, mp, and characteristic triazole \(^1\text{H} \text{NMR}\) of hybrid compounds.

| Compound | Yield (%) | mp (°C) | \(^1\text{H} \text{NMR}\) triazole C–H | \(^1\text{H} \text{NMR}\) | \(^{13}\text{C} \text{NMR}\) |
|----------|-----------|---------|-------------------------------------|----------------|----------------|
| 8        | 57        | 247–250 | 8.86                               | 128.3          |
| 9        | 61        | 254–256 | 8.87                               | 128.4          |
| 10       | 80        | 263–265 | 8.84                               | 128.4          |
| 11       | 45        | 138–140 | 8.72                               | 128.8          |
| 12       | 46        | 100–102 | 8.14                               | 128.0          |
| 13       | 74        | 279–283 | 8.71                               | 133.5          |
| 14       | 62        | 262–264 | 8.73                               | 133.4          |
| 15       | 50        | 238–240 | 8.73                               | 133.4          |
| 16       | 46        | 142–144 | 8.70                               | 133.4          |
| 17       | 47        | 112–115 | 8.71                               | 132.9          |

### Scheme 1. Reagents and conditions: (i) CuSO\(_4\)-5H\(_2\)O, sodium ascorbate, either H\(_2\)O/tBuOH (1:1 v/v) or H\(_2\)O/THF (1:1 v/v), 80 or 60 °C, overnight (ACR = 9-acridinyl; CHR = 2H-chromen-2-one-3-yl).
_results and discussion

Chemistry

9-Azidoacridine (1) was prepared by nucleophilic substitution of 9-chloroacridine in the presence of sodium azide under dry conditions,[25] and 3-azido-2H-chromen-2-one 2 was prepared through cyclisation, hydrolysis, and diazotisation of 2-hydroxybenzaldehyde as previously described.[26] The propargyl derivatives (3–7) were prepared by conventional alkylation through the reaction of the active nuclophile centres with propargyl bromide substrate[12,33]. The hybrid compounds (8–17) were synthesised by click reaction through cycloaddition of the respective azides (1–2) with propargyl derivatives (3–7) using copper (I) catalyst generated in situ via copper (II) sulphate reduction with sodium ascorbate (Scheme 1).[36]

For compounds 8–11 and 13–16, the reaction was performed by heating at 80 °C in the presence of 10 equivalents of sodium ascorbate in aqueous t-BuOH (1:1 v/v), while for compounds 12 and 17 the reaction was performed in aqueous THF (1:1 v/v). The final hybrid compounds were obtained in moderate to good yields with structure confirmation by 1H and 13C NMR, which identified the characteristic triazole signal (Table 1).

Biological evaluation

Preliminary in vitro anticancer screening

The hybrid compounds (8–17) were screened by the National Cancer Institute (NCI) (Bethesda, MD) in a primary anticancer assay in the full NCI-60 cell panel. The cell lines were derived from nine different cancer types: melanoma, leukaemia, colon, lung, CNS, renal, ovarian, breast, and prostate cancers. Compounds 11 and 12 having naproxen and valproic acid fragments in the acridine series as well all members of the set carrying the coumarin nucleus 13–17 showed either low or complete absence of cell growth inhibition against all cancer cell lines. However, compounds 8–10 of the acridine series having 6-methyluracil, uracil and phenytoin fragments displayed cell growth inhibition and selectivity against the different cell lines. Compound 8 displayed good to excellent cell growth inhibition against five types of cancer cell lines: leukaemia (K-562, HL-60 (TB), and SR), melanoma (LOX IMVI), ovarian (IGROV1), prostate (DU-145), and breast (MCF7) with percent growth inhibition of 83.7, 92.9, 88.6, 84.8, 67.0, 84.3, and 97.5, respectively. For compound 9, cell growth inhibition ranging from 61.3 to 67.0% was observed against K-562 and SR, MCF7 and T-47D cell lines. Compound 10 showed comparable cell growth inhibition percent ranging from 60.8 to 68.6 against K-562, SR, and MDA-MB-468; however, it showed marked selectivity and the best inhibitory activity of 84.1% against NCI-H522 (non-small cell lung cancer). Figure 3 provides a comparison of the percent growth inhibition of the cell lines on treatment with compounds 8–10.

MTT assay for cell viability

The cytoxicity of compounds 8–10 against MCF-7 breast cancer and DU-145 prostate cancer cell lines was determined using the MTT assay[36,47]. The compounds were all more cytotoxic in the MCF-7 cell line and in both cell lines the 6-methyluracil derivative (8) was more cytotoxic than the uracil derivative (9) with the bulkier phenytoin derivative (10) the least active (Table 2). Compound 8 displayed comparable cytotoxicity (IC50 2.70 ± 0.08 μM) with the standard doxorubicin (IC50 2.06 ± 0.04 μM), an anthracycline in clinical use as a wide spectrum antitumor agent, which exerts its action by intercalation between DNA double strands and blocks the topoisomerase II involved in the DNA replication process.[6]

Topoisomerase II B assay

The acridine compounds 8–10 were evaluated for inhibitory activity against MCF7 topoisomerase IIB and compounds 8 and 9 showed potent inhibitory activity with IC50 of 0.52 μM and 0.86 μM respectively compared with doxorubicin IC50 0.83 μM, while the phenytoin derivative 10 was less active with IC50 2.56 μM (Table 2).

DNA-flow cytometry analysis

Cell cycle parameters were compared for MCF-7 and DU-145 cell lines incubated for 24 h with the compounds 8–10 at their IC50 displayed in Table 2 and with vehicle (DMSO) as control. Cell cycle parameter for MCF-7 on exposure to compounds 8–10 showed induction of apoptosis as indicated by the increase in the percentage of apoptotic cells at the preG1 phase (24.4%, 16.3%, and 13.2% respectively compared with 2.3% for control) and decreased percentage of cells at G0/G1 phase (24.4%, 37.3%, and 43.5% respectively compared with 52.6% for control) and there was a concomitant increase in the cells at G2/M phase (48.3%, 39.8%, and 15.3% compared with 10.1% for control) (Table 3).

Similarly, exposure of DU-145 to compounds 8–10 increased the preG phase cell population (12.9%, 6.7%, and 4.5% respectively compared with 1.9% for control), whereas G0/G1 cells significantly reduced (34.6%, 41.5%, and 42.9% respectively compared with 49.1% for control) accompanied by a notable increase in the cells at G2/M phase (39.6%, 29.1%, and 20.9% compared with 13.7% for control). The percentage of the cells in S-phase showed no significant difference. Therefore, it seems compounds 8–10 inhibit the cell proliferation through cell cycle arrest at the G2/M phase, which consequently induces cell death by apoptosis. In line with the cytotoxicity results, apoptosis was more pronounced in MCF-7 and DU-145 cell lines treated with compounds 8–10 than those treated with control (Table 3).

Annexin V-FITC apoptosis assay

To allow for a precise estimation of apoptotic incidence induced by compounds 8–10, evaluation of the pro-apoptotic effect was performed by assay of the differential binding of the cells to propidium iodide (PI) and annexin V-FITC[48] that showed a notable effect on the percentage of apoptotic cells.
MCF-7 cells exposed to IC50 of compounds 8–10 revealed an increase in the percentage of annexin V-FITC positive apoptotic cells (UR+LR) 20.3% (10-fold) for 8, 13% (6.4-fold) for 9, and 10.7% (5.3-fold) for 10 compared with vehicle control (2.0%) (Figure 4). DU-145 cells showed a rise in the percent of annexin V-FITC positive apoptotic cells (UR+LR) 10.2% (sixfold) for 8, 4.3% (5.9-fold) for 9, and 3.6% (2 fold) for 10 compared with vehicle control (1.7%) (Figure 5). These results showed that the antiproliferative effect of compounds 8, 9, and 10 is due to their strong pro-apoptotic activity and further confirmed our findings on the sensitivity of the assayed cell lines to the tested compounds.

**Computational studies**

Computational studies of acridine hybrid derivatives 8–10 were performed using human topoisomerase IIβ in complex with DNA and amsacrine (PDB ID: 4G0U)40. The topoisomerase IIβ-ligand complexes were prepared by computational docking of 8, 9, and 10 the using MOE software41 and then subject to 200 ns MD simulations using the Desmond programme of Maestro44.

The 6-methyluracil ring on compound 8 is positioned between nucleic acid bases guanine (DG13) and cytidine (DC14) and forms a π–π interaction with DG13 and a H-bonding interaction between the 6-methyluracil NH and the carbonyl oxygen of the cytidine ring

![Figure 4](image-url) Effect of compounds 8–10 on the percentage of annexin V-FITC positive staining in MCF-7 cells on exposure for 24 h. The cells were treated with DMSO as control.

| Compound | Cell line | %G1  | %S   | %G2/M | %Pre-G1 |
|----------|-----------|------|------|-------|---------|
| 8        | MCF7      | 24.4 | 27.3 | 48.3  | 24.4    |
| 9        | MCF7      | 37.3 | 22.9 | 39.8  | 16.3    |
| 10       | MCF7      | 43.5 | 41.2 | 15.3  | 13.2    |
| DMSO     | MCF7      | 52.6 | 37.3 | 10.1  | 2.3     |
| 8        | DU-145    | 34.6 | 25.8 | 39.6  | 12.9    |
| 9        | DU-145    | 41.5 | 29.4 | 29.1  | 6.7     |
| 10       | DU-145    | 42.9 | 36.2 | 20.9  | 4.5     |
| DMSO     | MCF7      | 49.1 | 37.2 | 13.7  | 1.9     |
of DC14 (Figure 6(A)). The triazole ring of compound 8 also forms an H-bonding interaction between one of the triazole N atoms and the guanine NH of DG13, while the acridine ring is positioned parallel to the thymine ring of DT9 allowing formation of a π–π interaction. The complex is also stabilised by van der Waals interactions and hydrogen bonds with Val760, Gln778, Met781, Met782, Val785, Lys814, Ala816, Ala817, Pro819, and Ile822 (Figure 7(A)), with a binding affinity ($\Delta G$) of $-49.6 \pm 4$ kcal/mol.

In the case of compound 9, the anthracene is positioned closer to the major groove but between the nucleic acid bases adenine (DA12) and guanine (DG13) and forms a π–π interaction with DG13, while the uracil carbonyl oxygen forms a H-bonding interaction with DG13 and the triazole is positioned to form a π–π interaction with DT9 and also forms a water mediated H-bonding interaction between one of the triazole N atoms and the phosphate backbone of DT9 (Figure 6(B)). Although not as optimally positioned as compound 8, the uracil of compound 9 sits in a pocket composed of Glu417, Arg503, Gly504, Lys505, Asp561, and Ile565 stabilised by van der Waals interactions and hydrogen bonds, while the anthracene forms additional interactions with Gln778, Ala779, and Met782, resulting in a binding affinity ($\Delta G$) of $-35.9 \pm 3$ kcal/mol (Figure 7(B)).

The acridine moiety of the phenytoin derivative 10 is also positioned between the nucleic acid bases DA12 and DG13 forming a π–π interaction with DT12 and an edge to face π–π interaction with DC13 (Figure 6(C)). The phenytoin forms H-bonding interactions between one of the carbonyl oxygens and DG13 NH2 and DC14 cytidine ring, and the triazole ring forms an aryl-cation interaction with DG13. As seen with compound 9, the anthracene moiety of compound 10 sits in a pocket composed of Gln778, Ala779, Met781, Met782, and Met782 (Figure 7(C)). The phenytoin derivative (10) has an improved binding affinity ($\Delta G$) of $-67 \pm 5$ kcal/mol compared with the 6-methyuracil (8) and uracil (9) derivatives, which can be explained by the positioning of the bulky phenytoin group in a much wider pocket perpendicular to the acridine moiety, but away from the binding site, to minimise steric clashes (Figure 8).
Conclusions

The designed three component hybridised acridine and coumarin derivatives were synthesised and evaluated for their in vitro cancer cell growth inhibition activity. Among the acridine series compounds 8, 9, and 10 showed significant growth inhibition (61–97%) against six cancer cell lines. DNA interaction and topoisomerase inhibition properties of the planar tricyclic acridine ring were also investigated. 

Figure 6. 3D representation of the binding mode (A) compound 8 (green), (B) compound 9 (orange), and (C) compound 10 (gold). DNA structure (teal), protein (grey), and water molecules (red spheres).
Figure 7. Ligand interactions of final frame after 200 ns simulation of the topoisomerase IIB–ligand complexes of (A) compound 8, (B) compound 9 and (C) compound 10.
inducing cell apoptosis were demonstrated by flow cytometry and pro-apoptotic cell accumulation via the differential binding of the cells to annexin V-FITC and PI of MCF7 and DU-145 cell lines. Compounds 8, 9, and 10 showed cell growth inhibition at the micromolar level. MCF7 and DU-145 exposed to the most active compound 8 revealed IC₅₀ values of 2.7 and 26.1 µM respectively, which are comparable with the reference doxorubicin IC₅₀ values of 2.0 and 14.2 µM, respectively. DNA topoisomerase type IIb is involved in control of DNA replication and chromosome segregation during the normal cell cycle. Two new compounds 8 and 9 showed topoisomerase IIb inhibition potential with IC₅₀ of 0.52 µM and 0.86 µM respectively in comparison to the reference drug doxorubicin IC₅₀ of 0.83 µM. Computational studies illustrated the optimal positioning of the 6-methyluracil derivative 8 for intercalation with the DNA bases DG13, DC14, and DT9 with the 6-methyluracil and anthracene moieties respectively with additional protein–ligand interactions to stabilise the ligand in the binding site. The uracil derivative 9 is orientated in the opposite position with the anthracene positioned to intercalate with DG13 and in this case the triazole is positioned above DT9. The anthracene of the bulky phenytoin is positioned between DA12 and DG13, the phenytoin does not interact with DT9 and owing to steric limitations is extended away from the binding site with more limited protein–ligand interactions. The difference in binding interactions determined by the ligand conformation resulted in variances in cell growth inhibition and cytotoxic activity that can explain the depletion of anticancer activity of the other members of the prepared series 11–17. Owing to the promising results for the acridine derivatives with substituted pyrimidines, this pharmacophore can be considered as a lead for optimisation.

Acknowledgements

Molecular dynamics simulations were undertaken using the supercomputing facilities at Cardiff University operated by Advanced Research Computing at Cardiff (ARCCA) on behalf of the Cardiff Supercomputing Facility and the HPC Wales and Supercomputing Wales (SCW) projects.

Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

The authors acknowledge support of the latter, which is part-funded by the European Regional Development Fund (ERDF) via the Welsh Government.

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