# The Search for Novel Cancer Therapies: Catalytic Inhibition of Topoisomerase II by Substituted 9-aminoacridine Derivatives

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## Topoisomerase II is a Major Target of Lung Cancer Therapy

- Cancer is the leading cause of death in the developed world, claiming over 7.6 million lives in 2008 alone.
- Lung cancer is one of the most common cancers and has one of the highest mortality rates, accounting for nearly 18% of all cancer-related deaths each year.[1]
- Lung cancer predominantly occurs in two forms, small cell lung cancer (SCLC)(20%) and non-small cell lung cancer (NSCLC)(80%).
- Research into therapies to combat lung cancer has revealed an effective drug target: the enzyme topoisomerase II (topoII).
- Present drugs that inhibit topoII are called topoII poisons and stabilize the topoII-DNA covalent complex, inducing DNA damage and cell death.
- TopoII poisons have a major drawback: they are very toxic and can lead to chemotherapy-related leukemia in patients.
- This has prompted the search for drugs of similar action with reduced toxicity, which led to the discovery of the catalytic inhibitors.
- Catalytic topoII inhibitors interfere with topoII's function without inducing DNA damage by acting either before the DNA is broken or after it is rejoined.
- Recently, a small library of substituted 9-aminoacridines was discovered that have been shown to be catalytic inhibitors of topoII.
- In this study, we tested these compounds' antiproliferative abilities in human lung cancer

## Chemical Structure of Experimental Compounds

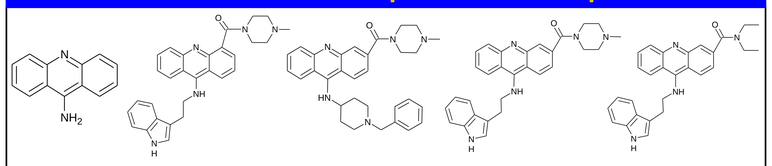
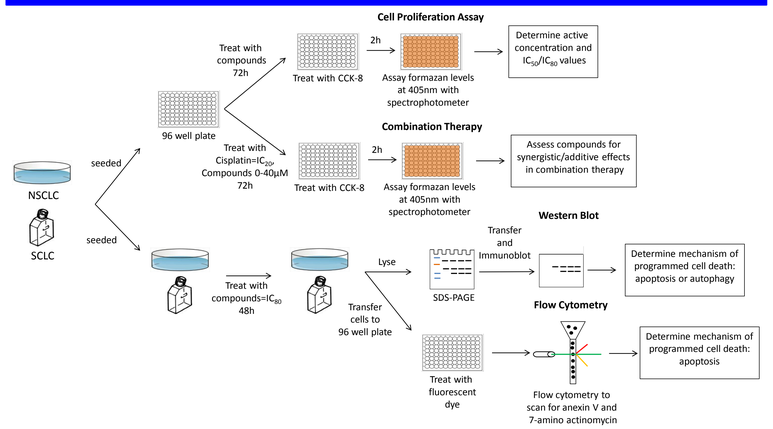



**Fig.1-** Chemical structures of substituted 9-aminoacridine derivatives shown alongside the parent structure of 9-aminoacridine.[2]

## Research Objective

**The objective of this research is to test the antiproliferative effects of four novel substituted 9-aminoacridine derivatives on lung cancer and to determine their viability as potential therapeutic agents for use in a clinical setting.**

## Methods




## Catalytic Cycle of Topoisomerase II

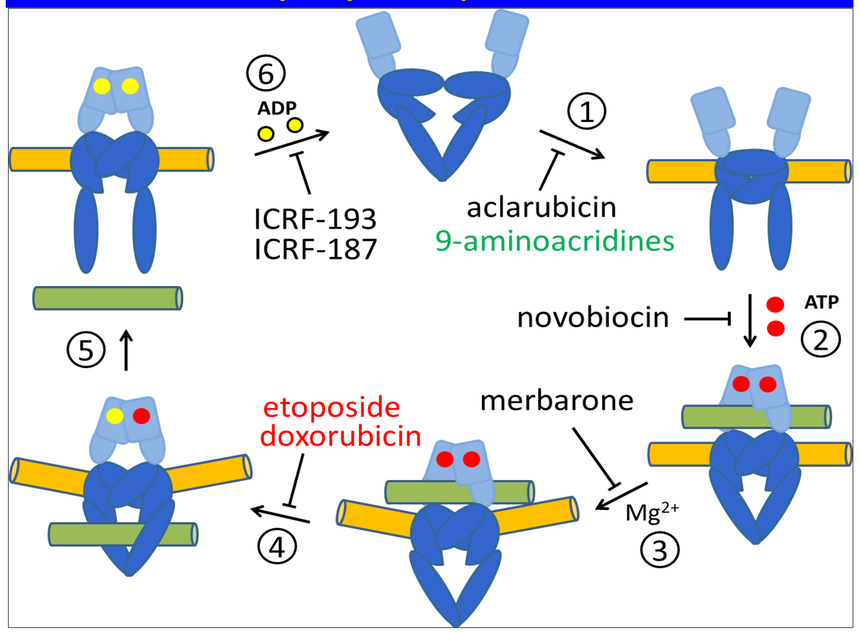



**Fig.2-** The catalytic cycle of DNA topoisomerase II. Light blue – ATPase domain, dark blue – core domain, yellow – G-segment, green – T-segment. Free topoII binds to the G-segment of duplex DNA (1). ATP (red circle) binds to the ATPase domains promoting T-segment capture and ATPase dimerization (2). N-gate closure induces G-segment cleavage and hydrolysis of one ATP (3). The DNA-gate opens and the T-segment passes through the G-segment (4). Hydrolysis of the second ATP religates the G-segment, the C-gate opens, and the T-segment dissociates (5). Both ADPs dissociate from the ATPase domain, the N-gate opens, the C-gate closes, and topoII dissociates from DNA (6). Inhibitors and their inhibition points are shown. Red – topoII poisons, black – catalytic inhibitors, green – drugs under evaluation. [3-6]

## Compounds Exhibit Significant Antiproliferation Activity

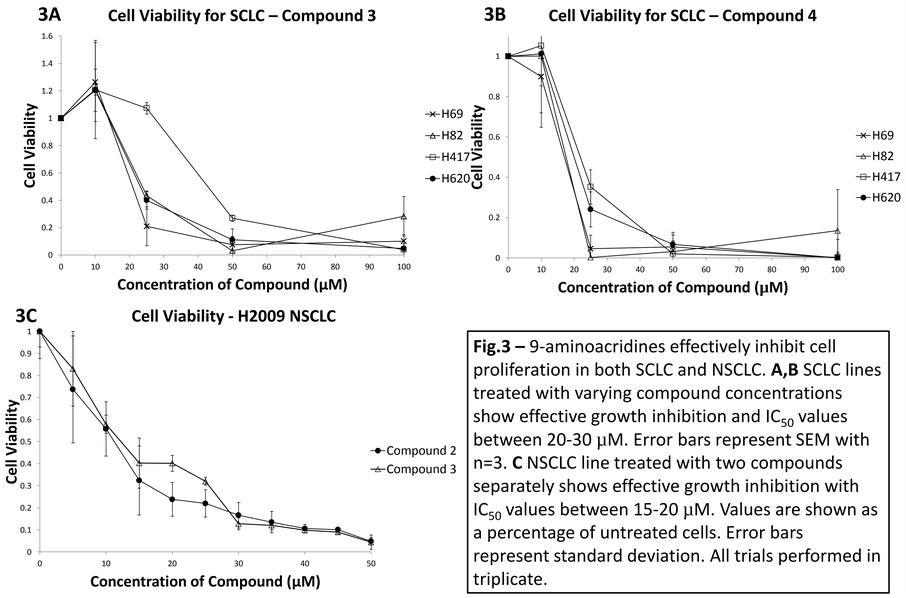



**Fig.3 –** 9-aminoacridines effectively inhibit cell proliferation in both SCLC and NSCLC. **A,B** SCLC lines treated with varying compound concentrations show effective growth inhibition and $IC_{50}$ values between 20-30 μM. Error bars represent SEM with n=3. **C** NSCLC line treated with two compounds separately shows effective growth inhibition with $IC_{50}$ values between 15-20 μM. Values are shown as a percentage of untreated cells. Error bars represent standard deviation. All trials performed in triplicate.

## TopoII Inhibitors Induce Both Apoptosis and Autophagy

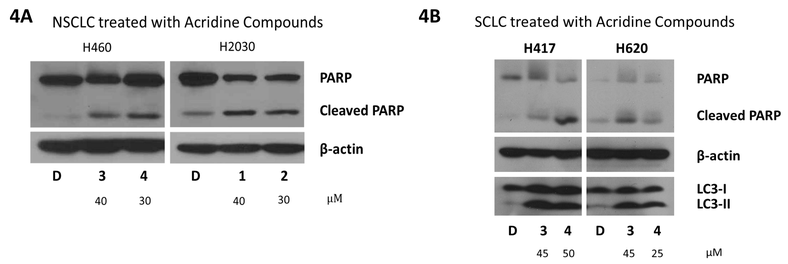



**Fig.4-** Immunoblot Analysis following 48h incubation with acridine-based compounds at $IC_{80}$ concentrations. **A** PARP cleavage in NSCLC cell lines indicate cells undergo apoptosis. **B** PARP cleavage in SCLC cells and significant LC3-II modification indicate that cells undergo both autophagy and apoptosis. β-actin was used as a loading control. D=100% dimethyl sulfoxide, the vehicle.

## Combination Therapy Shows Additive Effects with Cisplatin

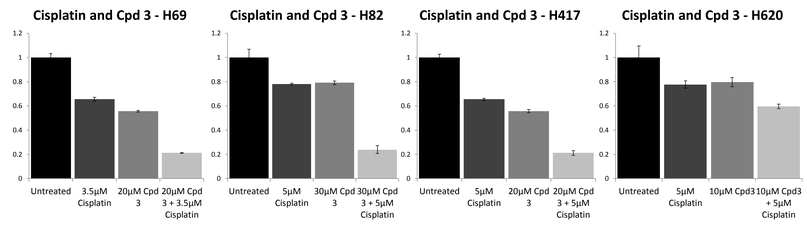



**Fig.5 –** Combination therapy shows additive effects in SCLC in most, but not all, treatment conditions. Error bars represent standard deviation. All trials performed in triplicate.

## Conclusions and Future Directions

- Substituted 9-aminoacridine compounds exhibit significant anticancer activity in human lung cancer *in vitro*.
- The potency of compounds as antiproliferative agents is greater in NSCLC lines than SCLC lines, showing a greater ability to inhibit cell growth and division.
- Compounds display differential potency with compounds ranked in decreasing order of potency from 4 > 3 > 2 > 1.
- NSCLC lines undergo apoptosis when treated with acridine compounds.
- SCLC lines undergo both autophagy and apoptosis simultaneously when treated with acridine compounds.
- Combination therapy with cisplatin shows additive drug effects with compound 3 showing the greatest effect.
- Future directions
  - Further studies are needed to understand the mechanisms behind the difference in programmed cell death observed between SCLC and NSCLC cancers.
  - *In vivo* studies should be initiated to determine drug activity and toxicity in animal models.
  - The dosage of compound necessary for activity in humans is unlikely to be achieved *in vivo*. However, further drug refinement through chemical modification to increase potency may lead to improved therapies in the future to combat this disease.

## References

1. Jemal, A. et al. Global cancer statistics. *CA: A Cancer Journal for Clinicians* n/a-n/a (2011).doi:10.3322/caac.20107
2. Goodell, J.R. et al. Acridine-Based Agents with Topoisomerase II Activity Inhibit Pancreatic Cancer Cell Proliferation and Induce Apoptosis. *J. Med. Chem.* 51, 179-182 (2008).
3. Collins, T.R.L., Hammes, G.G. & Hsieh, T. Analysis of the eukaryotic topoisomerase II DNA gate: a single-molecule FRET and structural perspective. *Nucleic Acids Research* 37, 712 -720 (2009).
4. Fass, D., Bogden, C.E. & Berger, J.M. Quaternary changes in topoisomerase II may direct orthogonal movement of two DNA strands. *Nat. Struct. Biol* 6, 322-326 (1999).
5. Larsen, A.K., Escargueil, A.E. & Skladanowski, A. Catalytic topoisomerase II inhibitors in cancer therapy. *Pharmacology & therapeutics* 99, 167–181 (2003).
6. Walker, J.V. & Nitiss, J.L. DNA topoisomerase II as a target for cancer chemotherapy. *Cancer Invest* 20, 570-589 (2002).