I. INTRODUCTION

This thesis deals with the synthesis, characterisation and evaluation of cytotoxic and antimicrobial activity of some novel heterocyclic compounds.

Cancer is a very dangerous disease characterized by the uncontrolled growth and spread of abnormal cells. Around the world, over 10 million cancer cases occur annually. Half of all men and one-third of all women will develop some form of cancer during their lifetime. It is one of the most feared diseases, primarily because half of those diagnosed with cancer will die from it. Cancer is a leading cause of death around the world, causing over 6 million deaths a year. However, it is now known that mainly treating with some chemotherapeutic agents can reduce the risk of developing many types of cancer. The knowledge about the cell cycle mechanisms and pathways in cancer pathogenesis is expanding rapidly with the recent developments of novel strategies and targets like Cyclin Dependent Kinases (CDK's), Oncogenic Human papilloma virus (HPV), Topoisomerase, Human papilloma virus, Cdc25 Phosphatase, ABC transporters, etc. Issues like target specificity and affinity must also be dealt for improving the success rates of drugs. In recent years, a continuous effort has been made both via computer aided drug design and reverse pharmacology using natural resources to discover and develop the drugs capable of specifically binding to the proteins/DNA responsible for cancer. So an attempt has been made to develop some novel synthetic heterocyclic compounds which are more potent and less adverse effect than the previous compounds already in the market.

Similarly many of the antimicrobial agents those already identified, which inhibits the growth of microorganisms such as bacteria, fungi etc. have produced many adverse
effects and are less potent. Therefore the need for new, effective and safe antimicrobial therapies is not likely to diminish in the near future. The discovery of such agents will undoubtedly demand the concerted, creative and innovative efforts of many individuals in a variety of disciplines.

Identification of promising anticancer(cytotoxic) and antimicrobial activities of many heterocyclic compounds like acridines, benzimidazoles, quinolines, isoquinolines, indoles etc. have stimulated considerable interest in the field of fused heterocyclic systems.

Thus, the synthesis of novel heterocyclic derivatives have attracted considerable attention. The explosive growth of heterocyclic chemistry is emphasized by the large number of research publications, monographs, and reviews.

From the above facts and prompted by the diverse biological activities, it is felt worthwhile to pay some attention on the synthesis of novel heterocyclic derivatives for their cytotoxic and antimicrobial activities.

The focus of my research work is on the development of novel heterocyclic derivatives, which might solve the problems associated with the known chemotherapeutic agents and offer advantages as target specific anti-cancer(cytotoxic) agents and/or antimicrobial agents. The present research project, therefore, aims at developing and evaluating such novel heterocyclic lead molecules for their cytotoxic activity and antimicrobial activity using suitable in vitro and in vivo models.

1.1. Acridine\(^{1,2}\)

Acridine was first isolated from anthracene fraction of coal tar in 1871 by Carl Grabe and Henrich Caro. It may also be known as benzoquinoline or dibenzopyridine. Since
19\textsuperscript{th} century they were first used as a raw material for the production of dyes and some valuable drugs.

![Acridine]

Their antiseptic activity has been discovered in the early 1900\textsuperscript{s} and some derivatives were extensively used during 1\textsuperscript{st} World War for their antibacterial and antimalarial properties. In the 1920\textsuperscript{s}, their potential in the fight against cancer was first noted. Since, a large number of acridine drugs, natural alkaloids or synthetic molecules have been tested as antitumor agents, a recent target being their telomerase and topoisomerase inhibition activity\textsuperscript{3}. At present a wide range of these compounds are used for the treatment of acute leukemia (amsacrine), as anticancer agents (ledacrine), as antibacterial agent (acriflavine and ethacridine), for action against parasites in the treatment of malaria, trypanosomiasis and leishmaniasis (quinacrine, acranil) and for treatment of Alzheimer’s disease (tacrine).

1.1.1. Synthesis of acridine

a) By oxidation of diphenylamines using lead oxide as the oxidizing agent.

\[
\text{N-\text{o-tolylbenzenamine}} \xrightarrow{\text{(O)}} \text{Acridine (i)}
\]

b) By passing the vapour of o-aminodiphenylmethane or benzylaniline through a red hot hot tube.

\[
\text{2-benzybenzenamine} \xrightarrow{\text{Heat} \ -2\text{H}_2} \text{Acridine (i)} \xrightarrow{\text{N-benzybenzenamine}}
\]
c) **Ullmann method.** This consists of heating o-chlorobenzoic acid with an arylamine in presence of copper powder to form an acridone which can be reduced into acridine by zinc dust distillation.

\[
\text{2-chlorobenzoic acid} + \text{aniline} \rightarrow \text{2-(phenylamino)benzoic acid} \rightarrow \text{acridine (i)}
\]

\[
\text{Cu, K}_2\text{CO}_3 \rightarrow \text{10,10a-dihydroacridin-9(8aH)-one}
\]

\[
\text{Zn dust}
\]

\[
\text{H}_2\text{SO}_4
\]

\[
\text{d) Goldberg method.} \text{ It is analogous to Ullmann method.}
\]

\[
\text{2-aminobenzoic acid} + \text{1-bromobenzene} \rightarrow \text{2-(phenylamino)benzoic acid} \rightarrow \text{acridine (i)}
\]

\[
\text{Cu, K}_2\text{CO}_3 \rightarrow \text{as in c}
\]

1.1.2. **Physical Properties**

Acridine and its homologues are stable compounds and weakly basic character. It is a colorless solid; melting point is 107 °C, possessing irritant vapour to nose and throat.

1.1.3. **Chemical properties**

As expected, acridine reacts with electrophilic reagents in benzenoid rings\(^1\). Thus nitration and bromination of acridine gives mainly 2,7-di- and 2,4,5,7-tetra- substituted products. Nucleophillic substitution takes place at position 9, e.g. acridine forms 9-aminoacridine when treated with sodamide. These results agreed with following \(\pi\)-electron densities, calculated on the basis of molecular orbital method, on the various carbon atoms.

\[
\begin{align*}
\text{Numbering of acridine molecule} & : 7 & 8 & 9 & 1 & 2 \\
& : 6 & 5 & 10 & 4 & 3 \\
\text{pi electron densities acridine} & : 0.695 & 0.939 & 0.999 & 1.014 & 1.906 & 0.0128
\end{align*}
\]
9-chloroacridine is readily substituted by nucleophilic reagents and their quaternary salt (readily obtained by alkylation) is even more reactive towards nucleophilic reagents than the parent compound.

1.1.4. Biological properties

Acridine nucleus is an important heterocycle present in a large number of biologically active compounds many of them are clinically used. Acridine derivatives exhibit a broad spectrum of biological activities including anti-microbial, antioxidant, anticancer, anti-malarial, anti-inflammatory, analgesic, antileishmanial, antinociceptive, acetyl cholinesterase inhibitors and anti-herpes etc.

1.2. Chalcones

Chalcone is a general term used for 1,3-diarylprop-2-ene-1-ones. They were widely distributed throughout the plant kingdom in the form of phenolic, enolic compounds. Chemically chalcones are diaryl α,β-unsaturated carbonyl compounds. Chalcones are important intermediate for the synthesis of various classes of organic compounds such as pyrazoles, isoxazoles, etc. They also exhibit versatile biological activity.

1.2.1. Synthesis of chalcones

Chalcones are prepared by Claisen Schimdt condensation method. Base catalysed condensation of appropriately substituted aldehydes and ketones possessing active α-hydrogens (acetophenone analogues).
1.2.2. Biological properties of chalcones

Recent studies on biological evaluation of chalcones has revealed some to be fumarate reductase inhibitor\(^6\), antileishmanial agents\(^6\), antimalarial\(^{16,15}\), antioxidant\(^{16}\), inhibition of nitric oxide production\(^{17}\), anticancer\(^{18,19}\), larvicidal\(^{20}\) and antimitotic\(^{21}\) etc.

1.3. Isoxazole\(^{22}\)

Isoxazole is a five-membered heterocyclic ring system with one nitrogen and one oxygen atom. The physical properties of isoxazoles and the presence of isoxazole nucleus in several important compounds led a special interest in the study of these compounds in a number of directions.

1.3.1. Synthesis of Isoxazoles

i. Isoxazoles may be prepared by the action of hydroxylamine on propargylaldehyde.

\[
\begin{align*}
\text{CHO} & \quad + \quad \text{NH}_2\text{OH} \quad \rightarrow \quad \left[ \begin{array}{c}
\text{HO} \end{array} \right] \quad \rightarrow \quad \text{Isoxazole}
\end{align*}
\]

ii. The reaction between 1,1,3,3-tetraethoxypropane and hydroxylamine hydrochloride results to isoxazoles.

\[
\begin{align*}
(\text{EtO})_2\text{CHCH}_2\text{CH}_2(\text{OEt})_2 & \quad \rightarrow \quad \text{Isoxazole}
\end{align*}
\]

1.3.2. Physical properties

Isoxazole is colourless liquid; boiling point is about 96 °C and smells like pyridine.

1.3.3. Chemical properties

Isoxazole is weakly basic. Isoxazoles, when substituted in the 3, 5-positions, are stable to alkalis, but when the 3-position is vacant, the ring is opened to form ketonitriles.
1.3.4. Biological properties

In recent years isoxazole and their derivatives have gained a lot of prominence due to their various biological activities such as antimicrobial\(^{23-25}\), anticancer\(^{26}\), anti-inflammatory\(^{27}\), analgesic\(^{28}\), larvicidal\(^{29}\) etc.

1.4. Pyrazole\(^{30-35}\)

Pyrazole is a five-member heterocyclic ring system with two nitrogen atoms. Our present knowledge of pyrazoles is largely due to knorr, who described the first member of this group in 1883. The physical properties of pyrazoles and the presence of pyrazole nucleus in several important compounds led a special interest in the study of these compounds in a number of directions.

1.4.1. Synthesis of pyrazoles

\(\alpha,\beta\)-unsaturated aldehydes, ketones or acids condensed with hydrazines to give pyrazolines which are then oxidised to pyrazoles by bromine or mercuric oxide.

1.4.2. Physical properties

Pyrazole is colourless solid; melting point is about 70 °C and crystallizes in long needles. Pyrazole is a tautomeric substance; the existence of tautomerism cannot be demonstrated in pyrazole itself, but it can be inferred by the consideration of pyrazole derivatives.
1.4.3. Chemical properties

Pyrazole is feebly basic and forms salts with inorganic acids; the imino hydrogen may be replaced by an acyl group. Pyrazoles are very resistant to oxidising and reducing agents, but may be hydrogenated catalytically, first to pyrazoline and then to pyrazolidine. Both of these compounds are stronger bases than pyrazole.

1.4.4. Biological properties

In recent years pyrazole and their derivatives have gained a lot of prominence due to their various biological activities such as antimicrobial\textsuperscript{36-37}, antioxidant\textsuperscript{38}, anticancer\textsuperscript{37}, anti-inflammatory\textsuperscript{39}, oestrogen receptor $\beta$ antagonist\textsuperscript{40}, antiviral\textsuperscript{41}, low-density lipoprotein(LDL) oxidation inhibitor\textsuperscript{42}, anti-pyretic\textsuperscript{43}, insecticidal\textsuperscript{44} and antitubercular activities\textsuperscript{45}.

1.5. Oxazine\textsuperscript{46}

Oxazines are six membered heterocyclic compounds containing one oxygen and one nitrogen. Many isomers exist depending on the relative position of the hetero atoms and relative position of the double bonds

A commercially available dihydro-1,3-oxazine is a reagent in the Meyers synthesis for aldehydes. Fluorescent dyes such as Nile red and Nile blue are based on the aromatic benzophenoxazine.
1.5.1. **Synthesis of oxazine**

1,3-oxazines were prepared by palladium–phosphine-catalyzed cyclo addition reactions of vinyloxetanes with heterocumulenes. 4-vinyl-1,3-oxazin-2-imines were obtained in fine yields by the reaction of 2-vinyloxetanes with carbodiimides in THF at RT for 12 hr using 1.5% Pd$_2$(dba)$_3$·CHCl$_3$ and 3% bidentate phosphine ligands when isocyanates were utilized in the reaction.

![Reaction scheme]

1.5.2. **Physical properties**

Oxazine is colourless liquid; boiling point is about 128 °C and smells like pyridine.

1.5.3. **Chemical properties**

A series of new substituted benzo[1,3]oxazines presenting bulky substituents on the chiral oxazine centre were prepared from isopropyl ketones or substituted cyclohexanones. Laser irradiation of these uncoloured compounds in solution promotes the cleavage of the C–O bond and the opening of the 1,3-oxazine ring generating a zwitterionic species, incorporating a 3H-indolium cation and a 4-nitrophenolate anion, that absorbs strongly at 440 nm. The photo generated coloured open isomers are thermally unstable and revert to the initial closed form with first order kinetics and lifetimes ranging from 13 to 68 hrs. These photochromic switches are extraordinarily stable displaying no significant degradation upon repetition of various irradiation/dark cycles.
1.5.4. Biological properties

In recent years oxazine and their derivatives have gained a lot of prominence due to their various biological activities\textsuperscript{47-53} like antimicrobial, anticancer, anti-inflammatory, analgesic and antitubercular activities etc.

1.6. Thiazines

These are six membered heterocyclic compounds with nitrogen and sulphur. They are found to be fairly stable. Thiourea has been used in the synthesis of heterocyclic rings containing nitrogen and sulphur\textsuperscript{54}.

1.6.1. Synthetic Methods

\(\alpha, \beta\) – Unsaturated ketones undergo cyclization with thiourea in presence of ethanol to yield 4, 6 – disubstituted compounds.

Hale and Brill\textsuperscript{55} reported the synthesis of 2-amino thiazine on heating 2-nitro propane diol with thiourea.

\[
\begin{align*}
\text{2-nitropropane-1,3-diol} & \quad + \quad \text{thiourea} \quad \rightarrow \quad \text{4,6-diphenyl-2H-1,3-thiazin-2-amine} \\
\text{5-nitro-2H-1,3-thiazin-2-imine}
\end{align*}
\]
Ghosh\textsuperscript{56} carried out the preparation of thiazine by intermolecular cyclization of various substituted propyl thiourea, 1-2 (2-Carbomethyl – 1) –2-thiourea derivative on heating in presence of acetic anhydride gave a good yield of 2-aryl amino –4, 4-dimethyl-6-keto thiazine.

Chase and walker\textsuperscript{57} reported the synthesis of 1, 3 thiazines from β ethylene ketones. This with thiourea in the presence of 48% hydrogen bromide kept for 8 days at room temperature yielded 2-amino-4, 6, 6-trimethyl-1, 3-thiazine in 78% yield.

1.6.2. Biological properties

Thiazine and their derivatives have gained a lot of prominence due to their various biological activities\textsuperscript{58-60} like antimicrobial, anti-inflammatory, analgesic and antimycobacterial activities etc.

1.7. Antimicrobial agents\textsuperscript{61-65}

The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria, could prevent the growth of another. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today’s common usage, the term antibiotic is used to refer to almost any drug that cures a microbial infection. Antimicrobials include not just antibiotics, but also synthetic compounds.


1.7.1. Antibacterial agents

An antibacterial agent may act by destroying the organism (bactericidal) or by inhibiting its growth (bacteriostatic). There are many category of antibiotics which are available in market such as penicillins, cephalosporins, tetracyclines, aminoglycosides, sulphonamides and some acridine derivatives such as acriflavine, ethacridine, quinacrine and acranil etc.

Even though lot of antibiotics are available in market the future effectiveness of antimicrobial therapy is somewhat in doubt. Microorganisms, especially bacteria, are becoming resistant to more and more antimicrobial agents. Bacteria found in hospitals appear to be especially resilient and are causing increasing difficulty for the sickest patients those in the hospital. In view of this selection has been made for the synthesis of some novel 9-anilinoacridines and evaluation of their antibacterial activity.

1.7.2. Antifungal agents

Fungal infections (mycoses) are widespread in the population; they are generally associated with the skin (e.g. 'athlete's foot') or mucous membranes (e.g. 'thrush'). In temperate climates such as the UK and in otherwise healthy people, they are mainly benign, being more of a nuisance than a threat. However, they become a more serious problem when the immune system is compromised or when they gain access to the systemic circulation. When this occurs, fungal infections can be fatal.

Currently marketed antifungal agents are broadly classified as naturally occurring antifungal antibiotics such as the polyenes, echinocandins and synthetic drugs including azoles and fluorinated pyrimidines. Because many infections are superficial, there are many topical preparations. Many antifungal agents are quite toxic and when systemic therapy is required these agents must often be used under strict medical
supervision. By considering the above fact, it has been planned to synthesize some novel 9-anilinoacridine derivatives which might have less toxicity when compared to currently available drug and evaluation of their antifungal activity.

1.8. Anticancer drugs

Cancer is a disease characterised by uncontrolled multiplication and spread of abnormal forms of the body's own cells. There are three main approaches to treat cancer that is cancer-surgical excision, irradiation and chemotherapy and the relative value of each of these approaches depends on the type of tumour and the stage of its development. Chemotherapy may be used on its own or as an adjunct to other forms of therapy.

1.8.1. Chemotherapy

The term chemotherapy was coined by Ehrlich himself at the beginning of the 20\textsuperscript{th} century to describe the use of synthetic chemicals to destroy infective agents. In recent years, the definition of the term has been broadened to include antibiotics substances produced by some micro-organisms (or by pharmaceutical chemists) that kill or inhibit the growth of other micro-organisms. Here, we broaden it still further to include agents that kill or inhibit the growth of cancer cells.

There are many category of chemotherapeutic agents are available in market which have distinct mechanisms of action which may vary in their effects on different types of normal and cancer cells. Among that one type of drugs act by inhibiting topoisomerase I/II these enzymes catalyze the inter conversion of different topological forms of DNA through concerted sequential DNA breaking-passing-resealing processes. The indispensable nature of the enzyme makes it the target of choice for antitumor agents. Some clinically available acridine derivatives such as amsacrine and
ledakrin act by the same above mentioned mechanism. Hence, in the present study focus has been on the synthesis of novel heterocyclic substituted 9-anilinoacridine derivative and evaluation of their *invitro* and *invivo* anticancer activity.

### 1.9. Human Topoisomerase II α

Human topoisomerase IIa (htopo II α)(Figure 1) is a homodimeric enzyme that requires ATP function. It is highly expressed in rapidly proliferating cells, and plays an essential role in replication, transcription and chromosome organization. It carries out a complex multistep reaction sequence on the genome involving a double strand break in one segment (G-segment) of the bound DNA substrate, passage of another duplex segment (T-segment) through the break and religation of the break to restore the continuity of the DNA backbone. The enzyme is believed to accomplish this difficult operation by opening and closing three different gates consisting of dimer interfaces at distinct points in the protein structure. The whole process and functioning of htopo IIa is controlled by the hydrolysis of ATP to ADP and inorganic phosphate which leads to an asymmetric retraction of Lys-378 which opens the enzyme-bridged middle gate of the protein- DNA complex and movement of the T-segment from the upper cavity formed in the ATPase domain into the lower cavity by the cleavage-reunion core.
1.10. Molecular Docking

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using for example scoring functions. The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism).

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Therefore docking is useful for predicting both the strength and type of signal produced. Hence docking plays an important role in the rational design of drugs.

Molecular docking techniques have shown great promise as a new tool in the discovery of novel small molecule drugs for targeting proteins. Fewer molecular docking studies have been performed targeting nucleic acids structures, despite advances in the understanding of the functional importance and the unique structural features of duplex, triplex, and G-quadruplex morphologies. This is unfortunate since not only are there clinically used drugs that target nucleic acids but also many forms of nucleic acids are becoming an increasingly attractive target for antineoplastic and antimicrobial agents. The few docking studies in which nucleic acids are targeted have focused on such sites as the minor groove of DNA, a tetra loop structure of RNA, and the major groove of an RNA duplex, while rarely targeting intercalation sites which
also hold therapeutic potential. The use of molecular docking has important implications for the synthesis and development of small molecule drugs that selectively target nucleic acids since these techniques have the potential to shed light on the interaction and mechanism of action of these ligands with targets that may have medicinal value.

Small molecules can interact with various morphologies of nucleic acids at multiple sites to alter nucleic acid function. In the case of duplex DNA, one drug class binds within the minor groove and a second class intercalates between existing base pairs of the nucleic acid structure. Intercalators and groove binders have distinctive thermodynamic signatures that indicate different driving forces for binding. The minor groove is a particularly attractive target for small molecules since this site has less competition from proteins and polymerases, which typically interact with the major groove. The closer proximity of the strands in the minor groove compared to the major groove allows more contact surface area for a small molecule to bind tightly. The unfavourable geometry of the major groove is another reason why few drugs target this groove.

1.9.1. Different softwares used for docking studies

Docking softwares

<table>
<thead>
<tr>
<th>Virtual screening</th>
<th>De novo design</th>
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<tr>
<td>Autodock</td>
<td>LUCI</td>
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<td>DOCK</td>
<td>GRID</td>
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<td>FlexX/E</td>
<td>MCSS</td>
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<tr>
<td>GLIDE</td>
<td>GOLD</td>
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<td>Surflex</td>
<td>GrowMol</td>
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PRODOCK, MC-DOK, Dock Vision, QXP, GLIDE are also some commonly used softwares for docking studies.

1.9.2. Overview:

The function of a major part of the genome is still unknown and the relationship between enzymes, hormones, signalling substances and various small molecules is still rather limited. It is expected that molecules such as proteins, synthetic organic molecules, small fluorescent dyes, inorganic complexes and ions can interact with DNA and interfere with DNA functions.

There are three important ways in which small molecules can reversibly bind to DNA, either by binding externally to the DNA-helix, or by intercalation between the base pairs, or by binding in the minor or major groove. Many types of drugs are known to bind to DNA through intercalation between consecutive nucleotides in the DNA strand. The notable achievements in this field consisting of fully synthetic DNA binding agents acridine groups, such as amsacrine, have raised a great interest in the synthesis and modifications of these compounds. Studies on pyrazoloacridines, imidazoacridinones, acridine carboxamides and triazoloacridinones are the most representative examples of the research in this area. These molecules have primarily been explored as chemotherapeutic agents (anticancer, antibacterial, antiprotozoal), because of the ability of the acridine chromophore to intercalate DNA (The acridine moieties are held in place by van der Waals forces supplemented by stronger ionic bonds to the phosphate ions of the DNA backbone) and inhibit topoisomerase and telomerase enzymes. It has been demonstrated by Hurwitz that the binding of acridine molecules interferes with normal DNA function by blocking the DNA starter required by polymerases to synthesise RNA and DNA and hence inhibits protein synthesis.
It seems reasonable to assume that, like for some other acridine derivatives, intercalation into DNA is not a sufficient condition for antitumor activity of these compounds. Rational design of new compounds of this chemotype requires knowledge about the structure and the interactions responsible for its stability.

Recently, it has been reported a novel non-cross linking platinum-acridine pharmacophore whose DNA interactions, unlike those of the “classical” conjugates, are clearly dominated by the non leaving intercalating moiety. Previous biochemical and biophysical studies have shown that platinum-acridine pharmacophore associates with dsDNA through a dual binding mode involving platinum binding to nucleobase nitrogen and intercalation of the acridine chromophore into the DNA base stack. The conjugate targets guanine (80%) and adenine (20%) bases at the base-pair steps 5’-CG/CG, 5’-TA/TA, and 5’-GA/TC, which are the preferred intercalation sites of free 9-aminoacridine derivative. Intercalation of the acridine moiety into the 5’-CG/CG base pair step on the 5’-face of the modified purine base is a feasible binding mode. Hence, in present study the focus is to synthesis novel 9-anilinoacridine derivatives and molecular docking studies where performed with Topoisomerase II.