Design & Synthesis of Novel Quinoxaline Small Molecules & Evaluation of their Biophysical & Nucleic Acid Binding Properties

Synopsis Submission to AcSIR

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Introduction

Nucleic acids are essential molecules that are characteristic of life on the earth. It stores and transfers hereditary information from one generation to next. Nucleic acids are basically divided into deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) as genetic material and it is involved in several biological processes like transcription, translation, gene storage. (Wang et al., 2016) The protection and conservation of genetic material are very crucial process and it preserves the cell from external damage. (Rodriguez et al., 2008). There are the number of proteins that involved in translation and replication of DNA. The small molecules which are mimicking this protein are modulating the DNA structure by binding with the DNA and inhibiting the replication process, sometime it may cause cell death. Hence the molecule targeting to nucleic acids play an important role as anticancer and antiviral. Therefore it is an important issue in recent year. (Shaikh et al., 2004)

Several Strategies have been devised for serving that purpose and revelation of "DNA/RNA targeting novel small molecules" remains a promising one. Till date, incessant research efforts have created such a pool of drugs and probable drug candidates with vivid poly functionalized basic scaffolds like quinoxaline. Quinoxaline and its related derivatives show various pharmacological and other relative bio-activities like antiproliferative (Montana et al., 2014; Desplat et al., 2010; Weng et al., 2011), antimicrobial (Henen et al., 2011; Vieira et al., 2014; Ishikawa et al., 2012), antiviral, (Waring et al., 2002; Wilhelmsson et al., 2008; Zeifman et al., 2014; Summa et al., 2012; Henen et al., 2011).

Statement of problem

Our idea of using monoquinoxaline moiety as a novel scaffold for targeting nucleic acid surfaced from the discovery of DNA interacting antitumor quinoxaline antibiotic such as Echinomycin. The structural template of the antibiotic includes two bicyclic chromophores (quinoxaline) connected across a dimerized cyclic peptide linker (Waring et.al 1974) i.e. depsipeptide. The structural complexity of this antibiotic and time consuming synthetic strategy motivated us towards the design and synthesis of monoquinoxaline based derivatives targeting nucleic acid. In our present research work we successfully designed and synthesised monoquinoxaline based small molecule targeting double stranded DNA (chapter 2). Subsequently we also derivatized the monoquinoxaline moiety with different functional groups and targeted towards HCV IRES (chapter 3). The modification of quinoxaline to dihydropyrimido fused quinoxaline ring system give rise to fluorescent analogue (chapter 4).

Methodology and Results

In second chapter part **2A** we showed, design and synthesis of 2,3 diamino derivatives of monoquinoxaline compound with benzyl substituent (**3d**, **3e**, **3f**) act as intercalator followed by DNA conformational change in sigmoidal fashion. (Mahata *et al.*, 2016) The design of quinoxaline derivatives was inspired by the structure of the dsDNA-bound quinoxaline antibiotic triostin A (**Figure** 1A) and the monoquinoxaline derivatives reported by Waring *et al.* The scaffold contained a triply substituted (C2, C3, and C6) monoquinoxaline (**Figure** 1B) with the C6-nitro group as a synthetic handle for C2/C3 substitutions, the C2/C3 quinoxaline amines as potential DNA hydrogen-bond donors, and the C3 propyl dimethyl amine for electrostatic interactions with DNA and to facilitate a snug fit in the minor-groove (alkyl chain). The C2-quinoxaline amine was substituted with various groups ranging from small and simple alkyl groups to bulky aromatic moieties (Mahata *et al.*, 2016).In quinoxaline antibiotics, diquinoxaline part of quinoxaline antibiotic (trostin A) help in the major grove interaction and peptide part help in the minor grove interaction , the design monoquinoxaline derivative the C2 part help in the minor grove interaction and C3 part help in the major grove interaction. On the other hand non benzyl based diaminoquinoxaline did not show intercalation property (*Angew. Chem. Int. Ed.*2016,**55**, 7733 –7736).

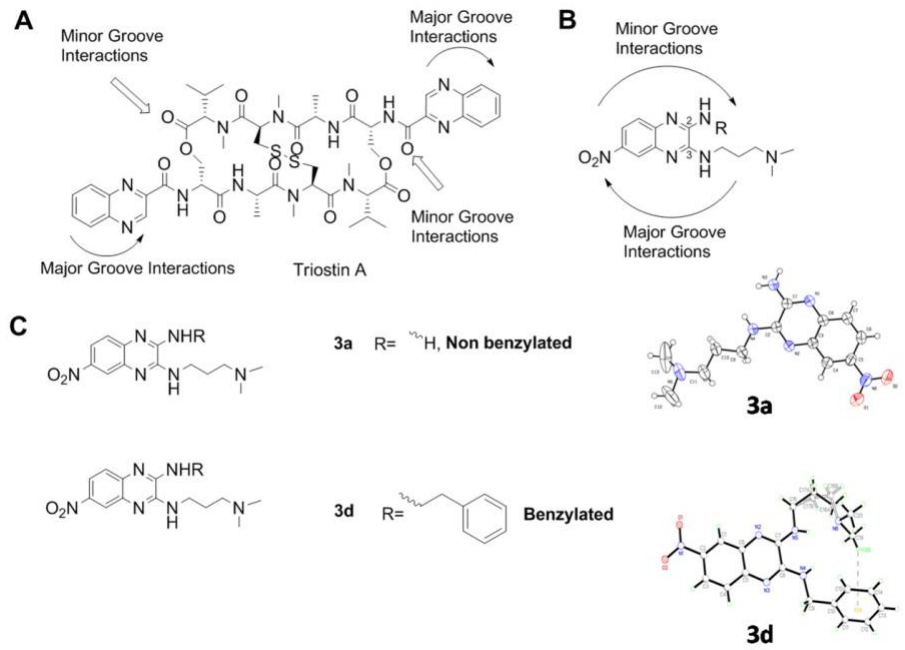


Figure 1: Design of quinoxaline based small molecule targeting deoxyribonucleic acid

We also suggest a model (**Figure** 2) where we shown how 3d molecule interact with DNA and 3d aggregate associated structural change in DNA, this model is supported by our DLS

and docking data. On the other hand non benzylated substituent not able to do intercalation associated subsequent event. Finally we conclude that benzyl substituted based quinoxaline derivatives excellent starting material for DNA based intercalator.

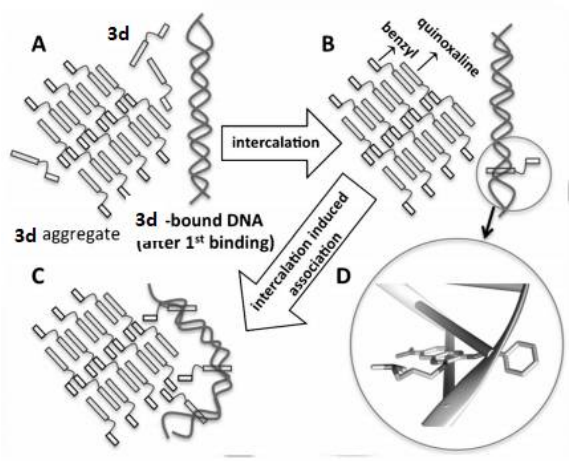


Figure 2 : A) As shown in picture firstly **3d** interact with DNA in non -intercalative mode followed by intercalation event by **3d** molecule A to B. C) Intercalation induced association of **3d** aggregate with DNA bound **3d** molecule B to C causing structural change in DNA.

However, without extensive and systematic changes of the benzyl group it was not clear whether aromatic stacking interactions or generic hydrophobic interactions are responsible for the observed sigmoidal binding.

In chapter 2B we showed Hydrophobicity of benzyl substituent is another factor that is associated with conformational change and condensation of DNA. Herein we have investigated DNA binding of **3d** (**Figure** 1) variants that contain various substituents on the benzyl moiety. Several analogues of **3d** were synthesized, where the benzylic moiety was replaced with 4-aminobenzyl, 4-bromobenzyl , 4-fluorobenzyl , 4-trifluoro methyl benzyl **,** 4 chloro benzyl and *N*-4-tolylpent-4-ynamide.

We also replaced the phenyl ring by pyridine and furan to study the effect of a heteroatom in the aromatic domain. We studied the interaction of benzyl varient from hydrophilic to hydrophobic with DNA by various biophysical techniques to probe the effect of DNA intercalation and DNA conformational change by varying the benzyl substituents of **3d**. Unwinding of supercoiled plasmid DNA upon DNA intercalation was tested by agarose gel shift assay. Circular dichroism studies of calf thymus DNA (ct DNA) with the compounds were performed to characterize the DNA binding mode and DNA conformational change. We further performed EtBr (a known DNA intercalator) displacement assays to complement the

gel shift assay and CD studies. On the basis observations we conclude that hydrophobic interactions between the benzyl substituent are the main influence behind DNA-binding induced conformational change. The implication of stacking interactions was testified using two substituents, pyridine and furan. Interestingly, none of them showed the characteristic DNA-binding signature, indicating stacking interactions might play a secondary role. This manuscript work is under preparation.

After successfully designed and synthesised benzyl substituent based small quinoxaline molecule targeting DNA, our next goal was designed and synthesis of quinoxaline based small molecule targeting IRES of hepatitis C virus.

In third chapter we showed the design and synthesis of quinoxaline based small molecule targeting IRES (Internal Ribosomal Entry Site) of hepatitis C virus. As from literature study it is well known fact that hepatitis C is highly contagious disease. It is estimated that millions of people have been diagnosed with this viral infection all over world and most of them are in chronic condition. In HCV genome most of conserve region is 5' and 3' untranslated region. The 5' region has an internal ribosomal entry site (IRES) and compound directed at IRES may surely benefit due to its unique and high sequence conserved structural domains and role in viral translation .

Design of monoquinoxaline based small molecule targeting IRES of HCV is based on crystal structure of subdomain IIa RNA inhibitor complex (**Figure** 3 section A) (Dibrov *et al.*). In crystal structure it shows that the benzimidazole part of ligand **1**(**Figure** 3 section D) help in stacking interaction with A53 and G52-C111. 2- amino and ring nitrogen form the Hoogsteen base pair with C58-G110 (**Figure** 3 section B). There is additional hydrogen bond formation of protonated *N,N* dimethyl propyl amine side chain interaction with phosphate back bone of A109 (**Figure** 3 section B). On the basis of crystal structure we designed monoquinoxaline based small molecule (**Figure** 3 section C), where we hypothesize that quinoxaline part help in stacking interaction with A53 and G52-C111. Quinoxaline and ring nitrogen might help in forming Hoogsteen base pair with C58-G110. Protonated *N,N* dimethyl propyl amine side chain would interact with phosphate back bone of A109. Here we were systematically changed the substituent's on second, third, sixth position of quinoxaline. The selected synthesised derivatives were showed significant inhibition of IRES mediated translation process which were analysed by dual luciferase assay. This work manuscript is also under preparation.

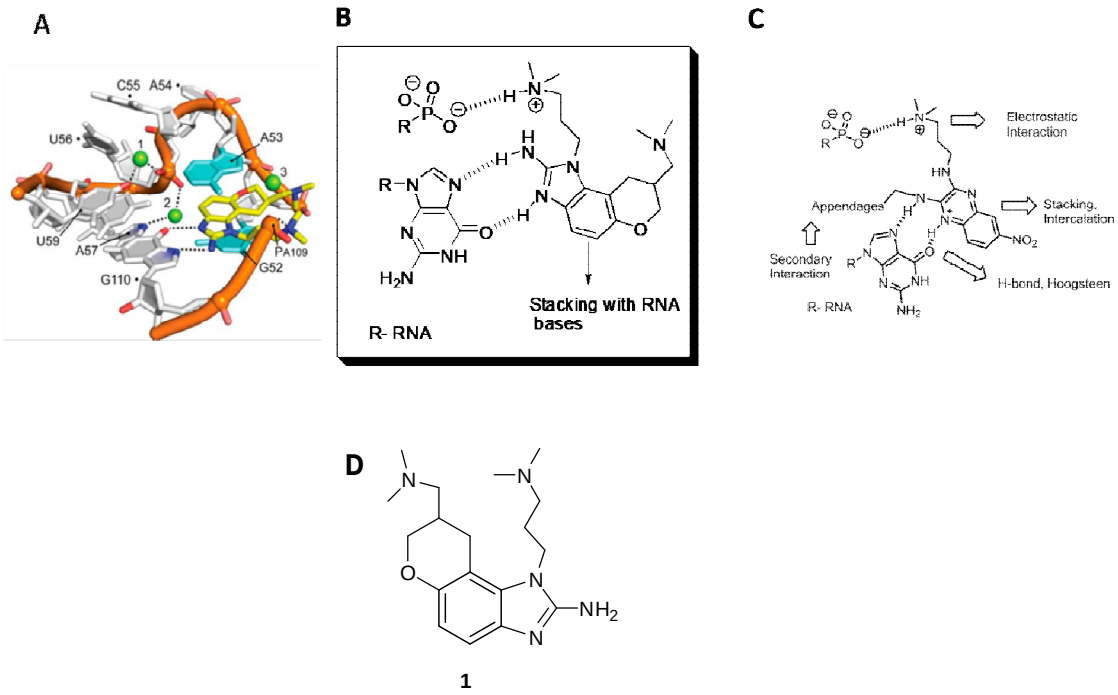
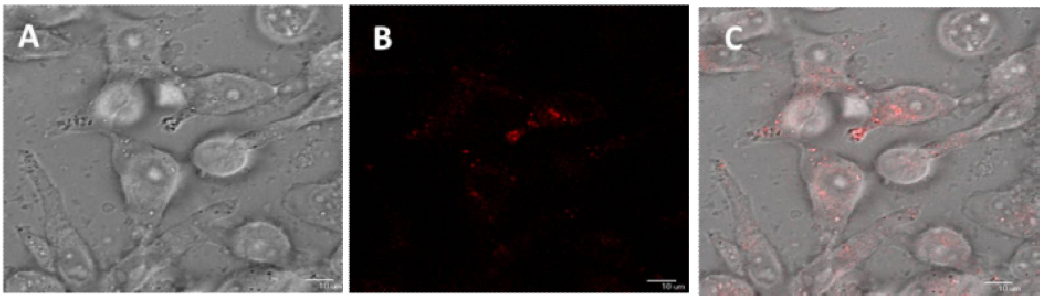
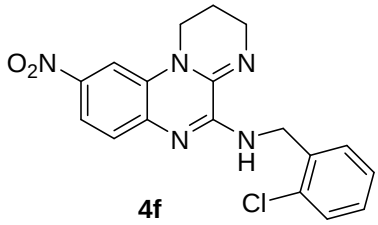


Figure 3: A) Crystal structure of subdomain IIa RNA inhibitor complex (Dibrov *et al.*, 2013) B) Imidazole and 2- amino nitrogen of ligand 1 forming Hoogsteen base pair with C58-G110 (Dibrov *et al.*, 2013) C) Designing of monoquinoxaline based small molecule based on crystal structure of subdomain IIa RNA inhibitor complex D) Structure of ligand 1.

Cellular imaging with fluorescent molecules is an important field and currently different fluorescent dyes are developed and marketed for their extensive use in the biomedical applications. We are also interested in development of fluorescent molecules with large stokes shift which can target biological macromolecules and can be imaged inside cells but 2,3 di-amino derivatives of benzylated monoquinoxaline compounds are non fluorescent, so our next goal is convert it into fluorescent analogues. In chapter four we showed novel reaction between 2,3 diamino derivatives of benzylated monoquinoxaline and ditertiary butyl acetylene dicarboxylate afforded fluorescent analogue (dihydropyrimido quinoxaline). Synthesis of dihydropyrimidoquinoxaline involves sp^3 C–N bond cleavage mechanism, which is not shown earlier with Quinoxaline compounds. We have also shown by laser confocal microscopy that live cell imaging of HepG2 cells can be performed with dihydropyrimidoquinoxaline compound and it is somewhere reside in cytoplasm of cell (**Figure** 4). (Kanungo *et al., 2015*). Further work with this scaffold derivatives is going on our lab and some of the derivatives of dihydropyrimidiquinoxaline have potential to target t-RNA with high fluorescent property. Extension work with dihydropyrimidoquinoxaline derivatives manuscript is under preparation.





4f

Figure 4: Fluorescent image of live HepG2 cells after incubation with **4f**. (A) Brightfield, (B) 1hr. with **4f** (50 nM), (C) DIC merged. Excitation and emission wavelength for **4f** was at 405 nm and 542 nm respectively.

List of Publications

Publications related to the present dissertation

1) **Kanungo, A**., Patra, D., Mukherjee, S., Mahata, T., Maulik, P.R., and Dutta, S. (2015). Synthesis of a visibly emissive 9-nitro-2, 3-dihydro-1 H-pyrimido [1, 2-a] quinoxalin-5-amine scaffold with large stokes shift and live cell imaging. RSC Advances *5*, 70958-70967.

2) Mahata, T., **Kanungo, A.**, Ganguly, S., Modugula, E.K., Choudhury, S., Pal, S.K., Basu, G., and Dutta, S. (2016). The Benzyl Moiety in a Quinoxaline-Based Scaffold Acts as a DNA Intercalation Switch. Angewandte Chemie International Edition *55*, 7733-7736.