IJPSR (2021), Volume 12, Issue 1 **(Review Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 January 2020; received in revised form, 16 April 2020; accepted, 18 April 2020; published 01 January 2021

G-QUADRUPLEX MOTIFS IN C MYC PROMOTER REGION AND THE ROLE OF VARIOUS SMALL MOLECULE LIGANDS/PROTEINS IN STABILIZING THIS PROMOTER REGION

Shikhar Tyagi *, Sarika Saxena, Nikita Kundu, Taniya Sharma and Sarvpreet Kaur

Amity Institute of Biotechnology, J3-Block, Sector-125, Expressway Highway, Amity University, Noida - 201303, Uttar Pradesh, India.

Keywords:

G-Quadruplex, c-myc, Proteins, Peptides, Ligands, Anti-cancer drugs

Correspondence to Author:
Shikhar Tyagi

PhD Research Scholar,
Amity Institute of Biotechnology,
J3-Block, Sector-125, Expressway Highway, Amity University, Noida - 201303, Uttar Pradesh, India.

E-mail: shikhar89@gmail.com

ABSTRACT: One of the essential scientific research concluded that DNA / RNA forms the hereditary or the genomic material in all life forms. The double-helical model given by Watson and Crick further added to the biological findings, and since then, various other DNA structures have been established *viz*., triple-stranded structures, quadruplexes, higher-order structures such as G-wires, polyads, *etc*. This review article takes into account the various aspects of the guanine rich called the G-quadruplex structure of the DNA, which are formed in the promoter region of a regulator gene called c-myc. Various ligands and proteins help in stabilizing/destabilizing the G-quadruplex structure present in the regulator gene, but a mutated version of the c-myc is seen during cancer, which leads to altered functions and expression of transcription protein leading to unregulated cell growth and proliferation. Thus, G-quadruplexes help in the regulation of the telomerase enzyme activity and can pave the way for the anticancer drugs, which are the need of the hour.

INTRODUCTION: One of the historical discoveries in the field of natural science during the last century includes the structure of B-DNA [1]. Watson-Crick gave the double-helix right-handed canonical form, but as the studies continued, various DNA sequences and structures were found, which led to the outcome of discovering other forms that existed and were different than the double helix. Various crucial roles are played by these unusual DNA structures in regulating the biological functions and also serve as an integral portion of the complex mechanism present in the living systems for regulation.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(1).22-43

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(1).22-43

Sequence-dependent conformational changes occur due to the negative supercoiling of the DNA, which leads to the formation of local DNA and other alternative conformations such as cruciforms, A-DNA, left-handed DNA (Z-DNA), triplexes, four-stranded DNA (quadruplexes) and others [2, 3].

Structure and Formation of Quadruplexes: Detailed studies conducted showed the existence of "tetraplex nucleic acids" (quadruplexes), which can originate from both DNA and RNA molecules. Sequences were having a high abundance of guanine (G-quadruplex), and cytosine (i-motifs) aid in the tetraplex structure specifically. However, other kinds of tetrads consisting of adenine [4], thymine [5,] and cytosine [6] or mixed tetrad containing Watson-Crick base-pairing were also demonstrated in the context of the formation of the G-quadruplex [7, 8]. GC and AT base-pairing showed that the presence of the G-tetrads was nil in the minor

groove of the quadruplex [9]. Cytosine rich sequences lead to the four-stranded secondary structures of DNA formation called i-motifs, and they are stabilized by acidic conditions. The two parallel stranded DNA duplex in the i-motifs are intercalated by cytosine-cytosine base pairing in an anti-parallel manner [10]. Even though there are unlimited forms in which the different sets of nucleotides can combine to give various and unique quadruplex structures, the most abundant and examined are the G-quadruplexes **Fig. 1**.

The adjacent run of guanine rich regions gives the best characterization of G-quadruplexes, and thus, they are classified as intramolecular and intermolecular structures based on the number of molecules involved [11]. Hoogsteen base pairing found in the guanine tetrads helps to stabilize the G-quadriplex structure by formation of hydrogen bonds **Fig. 1A** and the central channel possessing a negative charge is compensated by a monovalent metal ion (usually potassium or sodium ion) [12] however, the concentration of the DNA determines the stability and folding patterns [13]. A single nucleic acid strand or two or even four separate strands of DNA or RNA can form quadruplexes **Fig. 1B**. The diversified glycosidic bond angles result in the creation of different quadruplexes having chain orientations parallel or antiparallel [14].

Ribonucleosides have a glycosidic bond which confers anti geometry this leads to imparting of parallel conformation of RNA quadruplexes whereas, both the parallel and the anti-parallell forms are adopted by the DNA G-quadruplexes, and they have the capability to often switch from one to another, depending on experimental and sequence conditions [15]. A number of crucial factors affect the variety of G-quadruplex folding to a great extent, and these include the salt concentration, the position, and length of the loop, the abundance of different nucleotides in loops, and the number of tetrads [11]. Techniques such as CD spectroscopy, NMR spectroscopy, and X-ray have aided in the study of the detailed structure of G-quadruplexes **Fig. 2**.

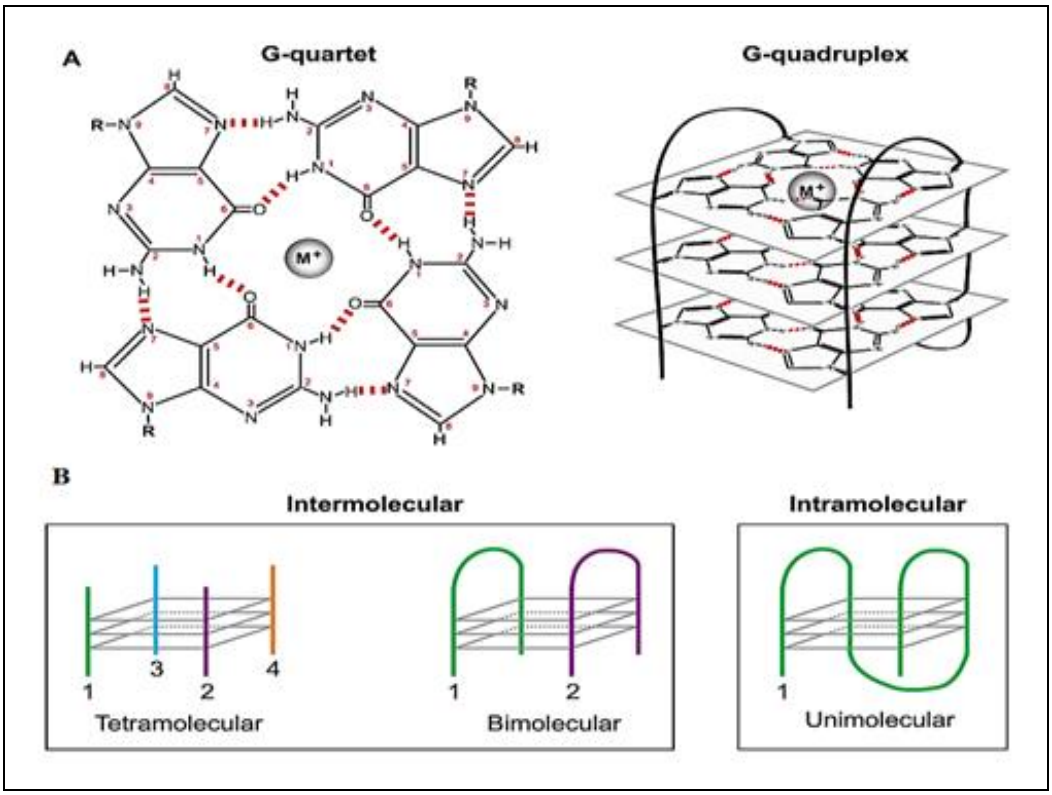

FIG. 1: (A) SCHEME OF HOOGSTEEN BASE-PARING IN G-QUADRUPLEX STRUCTURES. THE STACKED TETRADS OF GUANINES ARE STABILIZED BY A METAL ION (M+, RED) IN THE MIDDLE OF THE QUADRUPLEX; AND (B) QUADRUPLEXES CAN BE FORMED WITHIN A SINGLE NUCLEIC ACID STRAND, FROM TWO STRANDS (AS A DIMER OF HAIRPINS) OR FROM FOUR SEPARATE DNA OR RNA STRANDS. GREY SQUARE PLANES REPRESENT THE GUANINE TETRADS. GREEN, BLUE, PURPLE AND ORANGE LINES REPRESENT THE SUGAR-PHOSPHATE BACKBONE, WITH THE ARROWS SHOWING POLARITY OF THE NUCLEIC ACID CHAINS

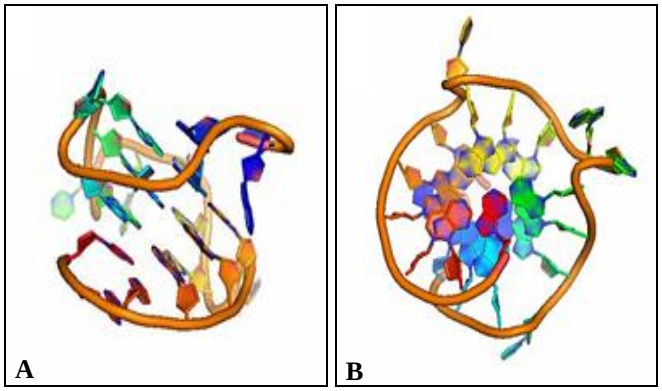

FIG. 2: STRUCTURE OF G-QUADRUPLEX IN THE NUCLEASE HYPERSENSITIVE ELEMENT (NHE) III1 REGION OF HUMAN c-Myc PROMOTER (PDBid: 1XAV, [16]). (A) SIDE VIEW; AND (B) BOTTOM VIEW. SUGAR-PHOSPHATE BACKBONE IS REPRESENTED BY THE ORANGE RIBBON, WITH THE GUANINE BASES FORMING THE TETRADS LOCATED IN THE MIDDLE

Presence of Quadruplex-Forming Sequences in Genomic DNA: Until 1962, the crystallographic methods did not determine the tetrameric arrangement of guanine bases as G-quartet even though since the late 19th century the guanosine self-association had been observed. The human telomeric sequence was the first discovered and characterized sequence, which formed the quadruplex [18]. *In-vitro* solvation of such structures helped in explaining that the promoters of oncogenes had the presence of G-rich sequences; subsequently, today, we have the power of sequencing the vast data which further allows the appropriate analysis of genomic sequences and provides us with the correct interpretation as to how quadruplexes are formed. Nowadays, due to the constant growing technologies, several tools have come up for the prediction of quadruplex formation [19-21]. The information regarding the composition and distribution in a nucleotide sequence capable of forming a putative quadruplex having G-rich motifs can be generated through various software programs developed; one such algorithm is the quadparser algorithm.

Based on the considerations like the stoichiometry of the strand, the number of stacked tetrads in the quadruplex core, presence of the base substitution or deletions, and also keeping in account the length and compositions of loops the algorithm is able to predict the quadruplex formation of a particular sequence. A total approximation of 3,76,000 putative quadruplex sequences has been given in the human genome [22,] and the frequent location in the promoter regions of oncogenes along with the telomeric and other G-rich repeats residing it suggests that they have a vital function in controlling the gene expression at the transcriptional level [19]. In addition, the presence of putative quadruplexes in an exon region predicts a role in translational regulation in RNA quadruplexes. Thus, it can be expected that the presence of quadruplex forming sequences is also there in the rich genomes of prokaryotes and other eukaryotes too and not only restricted in the human genome with potential significance in cell regulation and development [23, 24].

G-quadruplexes as Potential Anticancer Drug Targets: As the discovery of DNA G-quadruplexes was seen their recognition and the biological significance led to the intensified research and development into the G-quadruplex interactive compounds and approaching for an entirely novel cause, *i.e.*, drug targeting of secondary DNA structures represents an array for the anticancer drug design and development. First reports of exploring the therapeutic possibilities of targeting G-quadruplexes was given by Sun *et al*., in 1997 [25]. It was found that an enzyme called telomerase is seen as highly activated in most cancer cells and that the quadruplex ligands can inhibit the increased activity of telomerase enzyme.

In broader terms, the foundation for the existence of a mechanism of G-quadruplexes was laid by the subsequent discovery of the perylene derivative PIPER. It not only aided in driving the formation of G-quadruplexes [26] but also inhibited Sgs1-mediated G-quadruplex unwinding [27] and since then, the research for the development of better and enhanced agents is ongoing to identify and/or develop diverse families of small-molecule compounds with improved specificity and affinity. The completion of this vast objective cannot be attained just by a single route, and therefore, approaches involving *in-silico* and conventional screening methods need to go hand in hand along with the rational structure-based drug designing as well [28].

An important role is essayed by the structural data and its analysis in the design and development of G-quadruplex-interactive compounds. It is observed that there is a presence of a fused ring

system among the G-quadruplex- targeting ligands, and they are capable of stacking interactions with the terminal G-tetrads; thus, it seems to be a general feature of these quadruplexes. Moreover, the containment of the side chain substituents bearing the cationic charges in many of the ligand systems has the ability to interact with G-quadruplex grooves.

A ray of new avenues has open up with the constant and exponential growth in the number of papers concerning G4 in promoters, and it clearly reflects that the juncture is to turn on the opportunities of these potentials and deliver the requisitive results. Although there is a danger that the deeper we go in science sometimes the matter becomes more complex and we discover that there is a lot more to be studied and understood in order to gain clarity of the system and matter involved in the aspect thus, various studies on G4 structure, their stability, ligands interaction and their recognition, protein recruitment and so on are rapidly accumulating, and we hope to decipher the knowledge in its true sense. In the process of this review, the reader is going to have an insight into G-quadruplex formation in c-myc promoter region and will also learn the various ligands being discovered to interact specifically with the G-quadruplex motifs in the c myc promoter region as well. Merely knowing the facts is not the only concern but the readers will also get to know and understand about the role of these systems and how much physiologically importance do these mechanisms exert along with the mindset to understand the main issues concerning the correlations between the structural features of G4 formation in promoters focusing on c-Myc genes.

In recent years due to the serious threats to the human health posed by chronic non-infectious diseases and malignancies, the morbidity and mortality rates have been rising. Cancer, as we know, is a genetic disease, and it comprises a heterogeneous group of diseases, and hence, to develop effective drugs and therapy for it is challenging and interesting at its basal level too. It is clear enough that a single point source is not responsible for the onset of the disease; instead, multiple factors are generally contributing toward it, so the end result of many synergistic activities or sequential damages of DNA are involved along with the presence of several proto-oncogenes such as c-Myc that get activated. The down part is that the inbuilt counter DNA repair system involving the tumor suppressor genes too gets inactivated, leading to the alteration of the DNA system and no apoptosis regulation. Ultimately, the accumulation of these factors damages the DNA leading to cell transformation.

The Myc gene family, especially the c-myc has gained by far the greatest importance as it is found to play a crucial role as a transcription regulator, and its other vital functions are in the regulation of physiological processes such as cell cycle control, apoptosis, protein synthesis, and cell adhesion [29]. The myc family comprises of not only c-Myc but others like l-Myc and n-Myc and so on which mark their presence as well, but as the studies suggest and based on the data analysis so far, it is predicted that the over-expression of c-Myc is associated and/or is responsible for as many as 20% of human cancers. Imputable direct gene alterations caused due to the aberrant expression of c-Myc is likely to be associated with tumorigenesis and sustained tumor growth [30-32]. Thus, as a horizon of avenues, the inhibition of c-Myc has promised for the pathway of developing a therapeutic strategy for human cancer [33, 34]. In this review, we calculatingly try and understand the structural and functional features of c-Myc and also discuss the possible small molecule which acts as modulators of c-Myc along with highlighting their role as a promising aspect in developing anti-cancer therapeutics.

Structure of c-Myc: Isolation of the viral oncogene v-myc was from an avian retrovirus, and c-myc was discovered as a cellular homolog of the v-myc in human Burkitt's Lymphoma approximately 20 years ago [35-36]. Human c-myc **Fig. 3** consists of 3 exons and 2 introns found to be located on chromosome 8 q24.1 position [37]. It was found that the expression of human c-myc is de-regulated in cancer and further studies conducted on v-myc in chicken also led to the postulates that a large number of malignancies appear due to the altered function and expression of c-myc, these include mammary carcinoma, colon carcinoma, cervical carcinoma, myeloid leukemia, melanoma osteosarcoma, glioblastoma, and small-cell lung carcinoma.

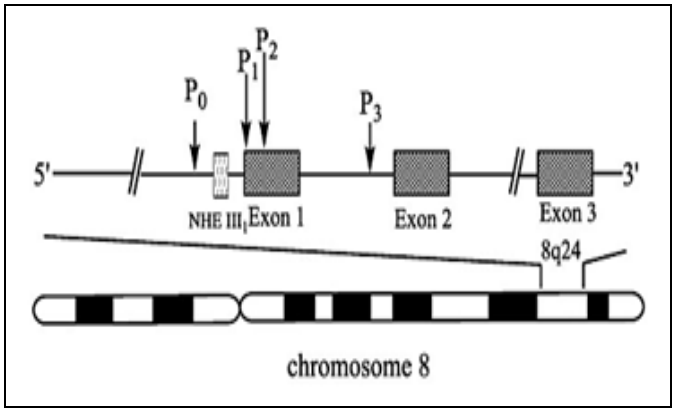

FIG. 3: A SCHEMATIC DIAGRAM OF HUMAN c-Myc GENE STRUCTURE. The diagram shows the location of exons and promoters indicated by arrows. The region of a combination of transcription activators with DNA, the nuclease hypersensitivity element III1 (NHE III_1), is located in the upstream of the P1 promoter.

The nuclease hypersensitivity element III1 (NHE III1), also known as Pu27 containing 27bp, controls 80-90% of the transcriptional activity although, it is reported that multiple promoters are responsible for the transcription of the c-Myc gene [38-39]. The P1 promoter at its upstream element has a guanine (G)-rich sequence, which is located at -142~-115 bp and is found to be transcriptionally active double helix structure **Fig. 4**. Intramolecular G-quadruplex structure is achieved by the G-rich strand, which contains repeated sequences having 3 or 4 guanine residues, and this help to suppress the c-myc transcription in a silenced form [41]. Hence, the element can prove to be a potential target in down-regulating the overexpression activity of c-Myc gene in tumor cells.

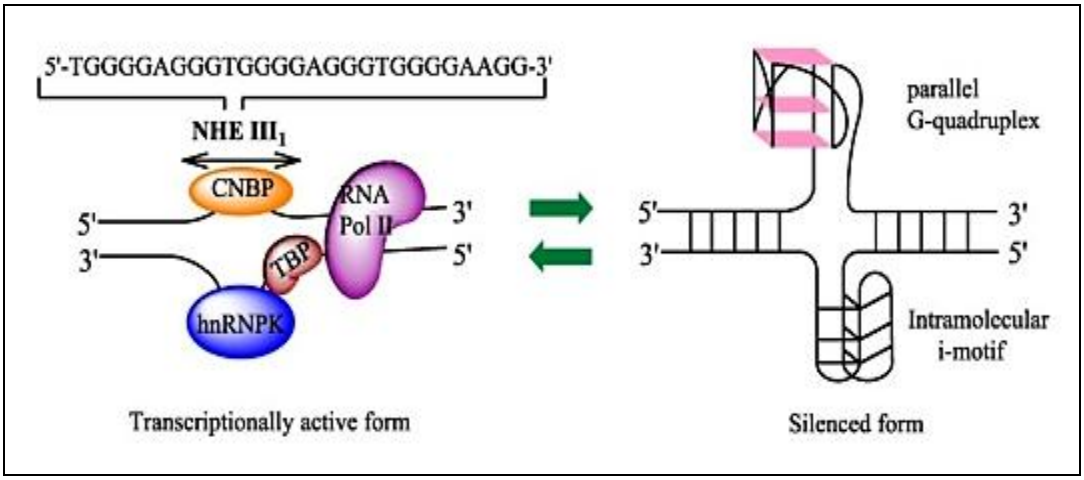

FIG. 4: THE DIAGRAM OF EQUILIBRIUM BETWEEN TWO FORMS OF NHE III_1 (NUCLEASE HYPERSENSITIVITY ELEMENT III_1). The left part represents a transcriptionally active form, which can regulate 80-90% of c-Myc transcription, and the right one a silenced form, with both G-quadruplex and i-motif structures being shown, that represses the transcription of c-Myc. CNBP: cellular nucleic acid-binding protein; hnRNP: heterogeneous nuclear ribonu-cleoprotein; TBP: TATA-box-binding protein; RNA Pol II: RNA polymerase II.

C-myc is a 65 kDa nuclear phosphoro-protein which belongs to the protein family of helix-loop-helix leucine zipper (b/HLH/LZ) structure as shown in **Fig. 5** [35-41]. We have understood the importance of c-myc protein, and it can be easily deciphered that considering c-myc protein as a master regulatory factor is apt as it plays pivotal roles in cell proliferation, metabolism, differen-tiation, and apoptosis thus, any dysregulation in human c-myc causes major and most common abnormalities found in cancer [42-47]. A total of 439 amino acids (aa) form the c-Myc protein, and it is divided into 3 regions consisting of an N-terminal, which acts as a transactivation domain (NTD), a C-terminal domain (CTD) and a central region. The transcription activation domain (TAD) is essential for the biological functions of the protein, and it is situated in the N-terminal, which is formed of three ~20 aa segments termed MYC box-I, II, and III (MBI, MBII, and MBIII) [47, 48]. The regulation of transcription and transformation processes are catered by MBI and MBII, which are located at aa 45-63 and 129-143, respectively. Further, the extension of amino acids from 360-437 form the C-terminal domain, and it plays a crucial role in the association with b/HLH/LZ-interacting proteins such as the Myc-associated factor X (MAX). C-myc, along with the myc associated factor X (MAX) leads to heterodimerization and, thus, has an essential role to play in the proliferation, transformation, and apoptosis [49, 50].

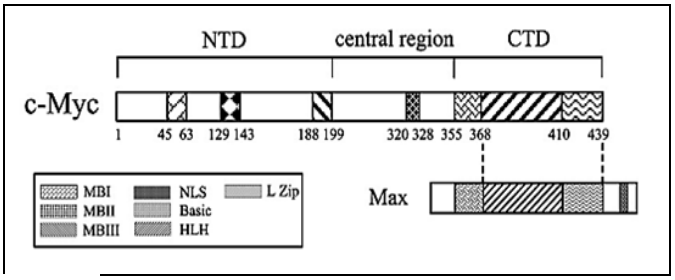

FIG. 5: **STRUCTURE OF HUMAN C-MYC PROTEIN.** The C-terminal domain (CTD) of c-Myc is pivotal for the association with MAX, a basic–helix-loop-helix (HLH)-leucine zipper (L Zip)-interacting protein. The N-terminal transactivation domain (NAD) consists of three elements, Myc box-I, II, III (MBI, MBII, and MBIII), which are the regulatory motifs necessary for c-Myc functions

Diverse Functions of c-Myc: The c-Myc oncoprotein is not only involved in various physiological functions such as cell cycle control, metabolism, protein biosynthesis and micro RNA regulation but additionally, the studies have also shown concrete evidence that about 15% of all human genes have been found to be regulating their expression due to the c-myc oncoprotein. Moreover, vital functions like cell apoptosis and senescence, DNA damage responses are also linked to the c-myc activity; if, overexpression of c-myc occurs, induction of DNA damage response is elicited. The elevated DNA damage response leads to the generation of reactive oxygen species and the formation of aberrant DNA-replication intermediates; hence, it can be stated that a dual role is portrayed in tumor progression, including tumor suppression and tumor maintenance by the DNA damage response [29, 40, 51, 52]. Thus, in this article, we try to reinstate the current trends and perspectives and functions of c-myc along with summing up and understanding the endogenous as well as inherently contradictory features of c-myc [52].

Regulation of Cell Cycle: Promoting the cell proliferation activity and arrestation of cell differentiation are the primary functions of c-Myc, but in addition, they are responsible for the regulation of the cell cycle in G1 phase transition [53-56, 61]. Studies have demonstrated the action of c-Myc in the activation and repression of cyclins D1 and D2, cyclin E, CDK4 (cyclin-dependent kinase 4), and cyclin B1 [50, 57-60]. In the G1 phase of cell cycle regulation of the cell proliferation is done by c-myc via induction of the cyclin D-CDK2 (cyclin-dependent kinase 2) activity [61, 62].

The segregation of p27Kip1 (cyclin-dependent kinase inhibitor 1B) from the cyclin D2-CDK4 complexes occurs once there is an activation of CDK4 and cyclin D2 [63]; however, under the critical conditions, the complexes can be phosphorylated by the CAK (cyclin activating kinase) where KIPI is dissociated with the cyclin E-CDK2 complexes [50]. The interaction of c-myc-Max heterodimer with transcription factors such as MIZ-1leads to the repression of CDK inhibitors P15 and P21 as the core promoter c-myc depicts itself as a repressor [64-68]. The coordination of growth factor-induced signaling pathways, such as NF-κB (nuclear factor κB), HIFs (hypoxia-inducible factors), *etc*., is required for the control of cellular proliferation and differentiation as it is a complex process; moreover, activation of c-Myc and cyclin D1 to promote cell proliferation is done by an important DNA-binding transcription factor NF-κB.

As discussed earlier, the role of transcription factors is important, and any alterations in the c-Myc can also lead to the repression of NF-κB transactivation and may act as an activator to further induce sensitivity to TNF (tumor necrosis factor) causing apoptosis [69]. It has also been noticed that direct targeting a putative transcription target such as cyclin D1or by indirect modulation of p21 and p27 by the HIFs they have the potential to alter cell-cycle progression. Thus, it would not be wrong to state that the c-myc has the capacity to act as a direct regulator of cell cycle regulation and the two α subunits of HIF-α, HIF-1α and HIF-2α respectively considerably inhibits cell cycle progression and increases proliferation by promoting and/or opposing c-Myc [70].

Ribosome Biogenesis and Protein Synthesis: It has been suggested that the coordination and the composite work of protein synthesis are regulated in multiple ways by the c-myc alongside acting as a cell cycle regulator. Many observations showed that the transcription of various RNA, iRNA, and ribosomes at the multi-level protein synthesis by the c-myc is controlled by the oncogene product [71]. Several studies have also lead to the results where overexpression of fibroblasts occurred at a much higher rate, *i.e.*, approximately three times superior to that observed in their parent lines during the protein synthesis in c-myc when cultured *in-vivo* [72].

The two very crucial factors include the regulation of transcription and ribosome biogenesis, which help in the mechanisms underlying the regulation of protein synthesis by c-Myc. The essential aspects such as synthesis and processing of ribosomal RNA and proteins are carried out by ribosome biogenesis, which itself is a process that is involved in many coordination steps [71], and until now, it has been proved that c-myc controls many genes of ribosomal proteins [57, 73-74]. Notably, it has been found that in ribosome biogenesis and translation, there is a requirement of nuclear RNA polymerase (RNA pol I and III), and it works well along with c-Myc as it has a coordination action with nuclear RNA polymerases [75-80]. The direct binding of TFIIIB (transcription factor IIIB), a pol III-specific transcription factor, and pol III-transcribed tRNA and 5SrRNA genes play a substantial role in the regulation of cell cycle progression and also, promote pol III transcriptions by c-Myc [61].

Enhanced protein synthesis is seen when stimulation of transcription attribute of rRNA (ribosomal RNA) is done by c-Myc [75, 76, 78] further, recent research showed that during tumorigenesis enhancement of protein synthesis occurs through transcriptional control along with other factor involving the activation of mTOR (the mammalian target of rapamycin) by c-Myc as an interwinding process of dependent phosphorylation of 4EBP1 (eukaryotic translation initiation factor 4E binding protein 1) occurs. Due to the esteem work role performed by the 4EBP1it has also been called as a master regulator since the protein synthesis control is carried out by it and the survival of the cancer cells in dependent tumor development is equally essential and done by c-myc [79].

Regulation of Stem Cell Functions: C-myc depicts its strong presence in the regulation and differentiation of stem cells and their function also, sufficient studies at various strata led to the point in deciphering the fact that with the cooperation of n-Myc c-Myc altogether is capable of inhibiting the differentiation of stem cells, such as embryonic stem cells [80] and neural stem cells [80-82] moreover, it checks and maintains their pluripotency and self-renewal capabilities. C-myc and n-myc in addition, are responsible for the regulation of the functions of hematopoietic stem cells [83].

Thus, the remarkable ability of c-Myc in regard to regulating the expression and function of stem cells is inseparably linked to its oncogenic activity.

Cell Apoptosis: C-Myc, as indicated, regulates cell apoptosis *via* signaling pathways, although the mechanism of apoptosis has not been fully understood and also it acts as an inducer of cell proliferation activity [84, 85]. However, according to the studies conducted, two major pathways have been proposed [85]. One of the signaling pathways suggests that expression of a tumor suppression protein such as ARF is found when an alternate reading frame of the INK4a/ARF locus is transcribed due to the induction by c-myc. Further, P53 (tumor protein p53) is activated when P 19Arf binds Mdm2 (mouse double minute 2); there is an activation of proapoptotic genes and cell cycle mediators , which in turn leads to enhancing the promotional apoptosis [84, 86-88]. The other mechanism puts forward the fact that regulation of apoptosis is done by c-myc via repressing the expression of anti-apoptotic proteins, such as Bcl-2 (B-cell lymphoma 2). This blocks the outer membrane permeability of the mitochondria and inhibits the release of cytochrome-c from mitochondria [89-91].

miRNA Expression: miRNAs are a set of small, non-protein-coding RNAs that have gained a lot of interest of the researchers in the recent times and the accumulation of various studies have provided with sufficient thoeries that c-Myc also regulates the expression of miRNA at the genetic level of expression at the post-transcriptional niche [92-97]. However, not much has been known as how the expression of miRNAs is regulated by c-Myc [98]. So far, varied explanations have been put ahead stating that many factors, such as SMAD (drosophila mothers against decapentaplegic) [99,100], P53 (tumor protein p53) [101], ATM (ataxia telangiectasia mutated) [102], MutLα (MLH1-PMS2 heterodimer) [103], and BRCAl (breast cancer 1) [104] have been able to show the modulation in the expression of miRNAs when an interaction occurs with microprocessor complexes. Although concrete evidence is yet to be brought in the science world, still it has been so proposed that through enhancing the expression of Drosha **Fig. 6** a c-Myc target gene is possible to indirectly promote the processing of miRNAs by c-myc [98].

Before any succinct compilation comes into existence, there is a need for proper manipulation of the c-Myc-miRNA complex that may lead to a novel therapy for malignancies.

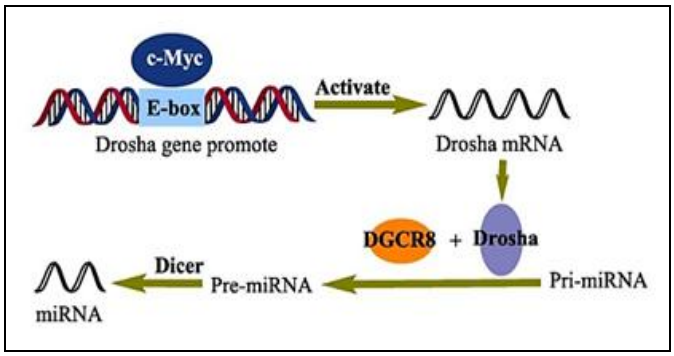

FIG. 6: A SCHEMATIC REPRESENTATION OF THE c-Myc REGULATION OF miRNAs BIOGENESIS. Directly interacting with the E-box of the drosha gene promoter, c-Myc activates the transcription of Drosha, which in turn promotes the biogenesis of miRNAs

Parallel-Stranded G-quadruplex Structure Formed in the Human c-MYC Promoter:

G-quadruplexes Formed in the c-MYC Promoter: Multiple G-quadruplex structures are formed by the G-rich sequence MycPu27 containing a 27-mer NHE III1 region. It is comprising of five consecutive runs of guanines - with three runs composed of four guanines each and two runs composed of three guanines each **Fig. 7A** [105]. It has been shown that the 5′-run of guanines (G-tract I) are not involved in the formation of the major G-quadruplex structure of Pu27, as depicted by the data obtained by Native gel EMSA and DMS foot-printing techniques [106]. Whereas, studies have given that the four consecutive 3′ runs of guanines (Myc2345) are a mixture of four loop isomers that are majorly responsible for G-quadruplex formation. The G14 and G23 in the loop region, have been found to be the predominant loop isomer that has been shown by using DMS cleavage and EMSA data [107]. Moreover, the major c-myc and G-quadruplex Myc2345 complex folding topology have been shown to be parallel-stranded [107, 108]. In addition, the parallel strand folding is also adopted by the minor G-quadruplex formed in Pu27 using the 1,2,4,5 G-tracts (Myc1245) [106] **Fig. 7B** [109].

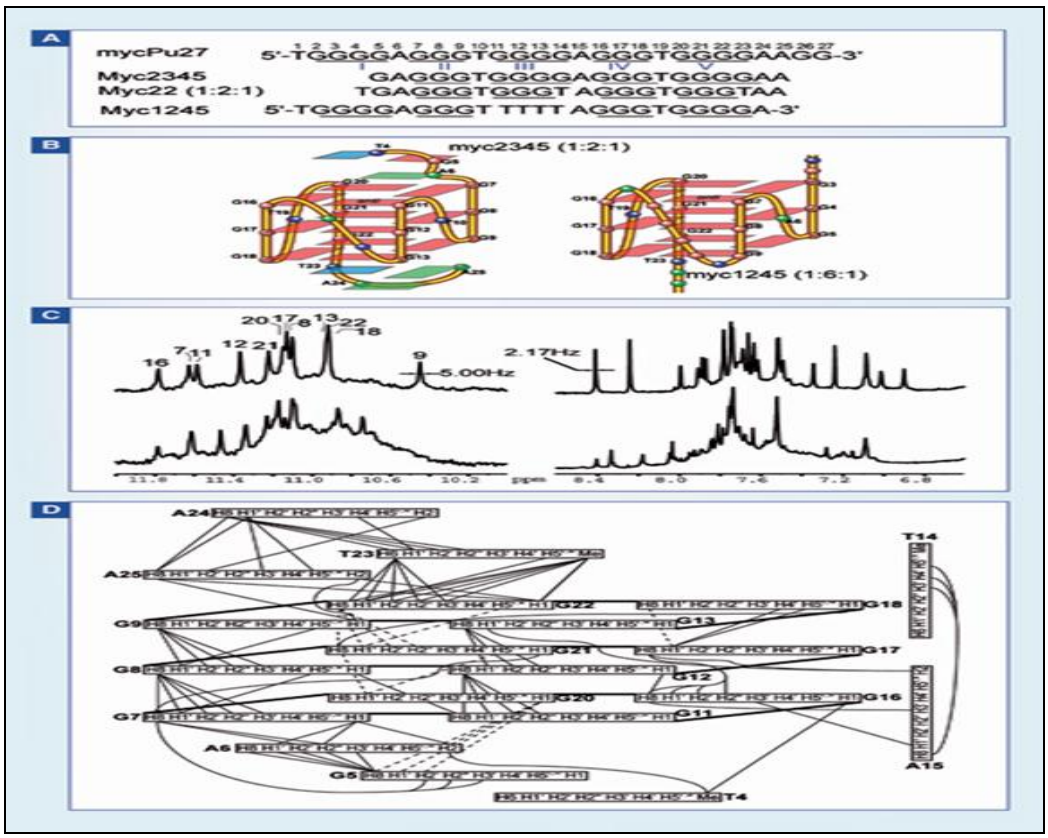

FIG. 7: (A) THE C-MYC PROMOTER SEQUENCE AND ITS MODIFICATIONS. (B) THE FOLDING STRUCTURE OF THE MAJOR G-QUADRUPLEX FORMED IN THE c-MYC PROMOTER, Myc2345 (1:2:1) (LEFT) AND THE MINOR G-QUADRUPLEX, Myc1245 (RIGHT). (C) 1D NMR SPECTRA OF THE T-Myc2345 SEQUENCE (BOTTOM) AND ITS MAJOR LOOP ISOMER Myc22 (TOP). (D) INTER-RESIDUE NOEs OF MYC22, WHICH FORMS THE MAJOR G-QUADRUPLEX OF THE c-MYC PROMOTER. THE NOE CONNECTIVITIES CLEARLY DEFINE THE QUADRUPLEX CONFORMATION AND PROVIDE DISTANCE RESTRAINTS FOR STRUCTURE CALCULATION

Molecular Structure of the Major G-quadruplexes Formed in the c-MYC Promoter: The c-myc containing the major G-quadruplex in the silencer element has structurally been explained by the scientist through the NMR technique **Fig. 7B**, it is formed in the promoter region of an oncogene and considered to be the first molecular structure of a G-quadruplex [109]. The restriction of the mixture of loop isomers is done by the sequence MYC22 having a specific region **Fig. 7A**. The composition consists of two G-to-T mutations at residue positions 14 and 23 of the wild-type c-MYC sequence, which helps the loop isomers to remain in a conformation that is predominantly single and forming a c-myc G-quadruplex complex in K^+ solution. In comparison to the wild type sequence of MYC22 the improved structure of MYC22 having far batter line width and resolution has been seen in **Fig. 7C**. The overall conformation defining the major c-myc G-quadruplex, Myc2345 complex structure, has been observed in 2D-NOESY comprising of many inter-residue NOE cross-peaks **Fig. 7D**. Based on the data generated from the restrained molecular dynamics(RMD) the various NOE connectivities were used to obtain a linkage which later led to the calculations and conclusion that a family consisting of 20 lowest energy input having well-refined structures were produced through this process with the average rms deviation being 0.88 Å including all atoms [109].

Based on the NMR studies, figure 8 showcases a representational structure of the major c-myc G-quadruplex formed by MYC22 in a refined solution structural existence. This structure serves to be a representative as it was first of its kind to glorify the molecular structure showing the parallel-stranded motif with a single-nucleotide double-strand-reversal loop (G3NG3) thus, it was taken and prevailed as a robust motif that appears to be ubiquitous in the G-quadruplex-forming promoter sequences. Four parallel DNA strands having three G-tetrads are linked by three double strands consisting of reversal side loops in the intramolecular Myc2345 G-quadruplex, it also includes a double-nucleotide loop and two single-nucleotide loops. The basis of width measurement is adopted by the four grooves of this G-quadruplex; an extended sugar backbone strand is observed while the thymine base when in solvent sticks out in a single nucleotide loop. Whereas, if the double nucleotide T14A15 loop is seen the extension of sugar backbone is not as much compared to the single thymine loop and the T14 base thymine sticks out while the adenine A15 points to the top of quadruplex where the G- tetrad groove is present with H2 at its end into the solvent.

The molecular structure explains the parallel single-nucleotide stranded motifs stability; two adjacent guanine strands make the 3'- end of one G-strand and they lie in proximity to the 5'-end of next G-strand while they are in the right-handed twist formation. Hence, this favors the single nucleotide double strand reversal loop **Fig. 8A**. Further, it was also concluded based on a variable temperature study that the single nucleotide loop has more stability than the double nucleotide loop; thus, the G16 and G13 the two ends of the TA loop are considered to be the first melting points of Myc2345 G -quadruplex [109].

Well-defined capping structures are located on both the sides of the Myc2345 G-quadruplex structure. A twisted backbone conformation is adopted by the 5′-TGA flanking strand, and stacking of the A6 residue is visible with the bottom tetrad's G7, beside this it was noticed that on the side of G7 and G20 lay the G5 residue **Fig. 9A**. The cap at the 3'-end of the c-Myc G-quadruplex adopts a well structured and defined fold back at the 3′-flanking TAA **Fig. 8B**. The top G-tetrad having G9 attaches with the terminal A25, and the folding back leads to the stacking between the two, whereas the formation of the Hoogsteen Hydrogen bonding is seen among A25 and the T23 base as it stacks on top of the G22 residue. It is also known that the wild type G23 led to the formation of the T23 as a result of the mutation, and the T23:A25 is stacked on top by the A24 residue. A stable fold back conformation is observed between the G-quadruplex and wild type 3'- flanking GAA sequence, and the molecular dynamics modeling showed a similar folding pattern between G23 and A25 bases via Hoogsteen hydrogen bonding [109] **Fig. 9C**. In a comparative study, we see that the melting temperature of wild type myc Pu27 is much less as to that of MYC22, which is over 85 °C. Myc Pu27 as the wild type c-MYC has its promoter DNA sequences extended as well as flanking; thus, they are able to adapt themselves for

the essential capping formations which further, help them to stabilize the G-quadruplex conformations. This quintessential capping structures of Myc2345 G-quadruplex may later be of utmost importance as it can provide potential binding sites for drug targeting.

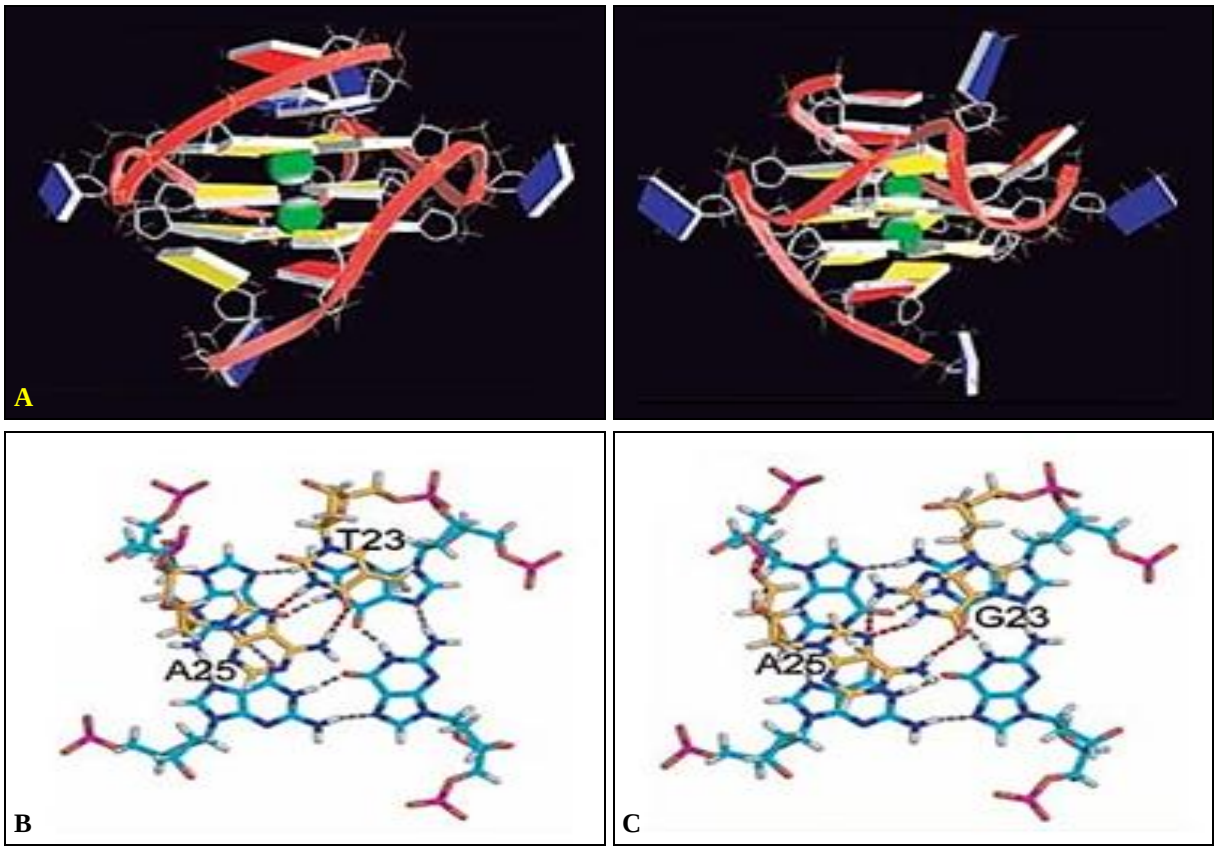

FIG. 8: (A) REPRESENTATIVE NMR STRUCTURE OF THE MAJOR c-MYC PROMOTER G-QUADRUPLEX FORMED BY Myc22, A PARALLEL-STRANDED STRUCTURE, IN TWO OPPOSITE VIEWS. TWO POTASSIUM IONS COORDINATED BETWEEN THE G-TETRADS ARE INCLUDED FOR THE CALCULATION AND ARE SHOWN AS GREEN SPHERES. (GUANINE: YELLOW; ADENINE: RED; THYMINE: BLUE) THE 3′ END VIEW OF THE G-QUADRUPLEX WITH MUTANT T23 (B) AND WILD-TYPE G23 (C) THE HYDROGEN BONDS OF THE TOP TETRAD (BLACK) AND THE T/G23: A25 BASE PAIR (GREEN) ARE SHOWN IN DASHED LINES

All the composites so far listed in the review have been able to bind the G-quadruplex structure selectively in comparison to other forms of DNA as, so suggesting that different G-quadruplex-interactive compounds can only selectively bind different types of G-quadruplexes; hence, it gives us the fact that this selectivity is likely to be related to their biological activity. For example, excellent *in-vivo* activity is displayed by a G quadruplex drug in various solid tumors named quarfloxacin® (CX-3543, Cylene Pharmaceuticals in San Diego, CA, USA) which is currently in Phase II clinical trials. Relative low cytotoxic measurements act as an important crusader in delivering various other G-quadruplex-interactive compounds that have come into play for becoming the prospective anticancer agents. A better understanding of the G-quadruplexes can be linked to the fact that the G-quadruplex-interactive compounds themselves have contributed immensely, and their build-up can be utilized for understanding G-quadruplexes as a therapeutic target.

Small-Molecule Ligands Targeting the c-Myc Promoter G-quadruplexes:

Advances in the Development of Anti-tumor Drugs Targeting the c-Myc Promoter G-Quadruplexes: Based on the indications G-quadruplexes have so far provided in regulation of c-Myc transcription process and in suppressing tumorigenicity they can be called as the future promising target for anti-cancer therapy. There is a G-rich sequence of c-Myc promoter known as NHE $(III)_1$, *i.e.*, Nuclease hypersensitive element and it has two differential forms present, one that is transcriptionally active and the other is silenced as

shown in **Fig. 4**. When the region is active, the G-rich sequence exists in the form of the double helix and along with the cooperation of RNA polymerses, and various other factors are able to transcribe c-Myc gene. Unlike the contrasting situation when in silenced form, prevention of interaction between the various transcription factors with the elemental form is done by the region containing the G-quadruplex structure, and this further leads to the down-regulation of c-Myc transcription [110]. However, it has been noticed that very few G-quadruplexex exist in the c-Myc promoter region under physiological conditions [7880]. Small molecule ligands that are specific and can lead to induction aiding in formation and stabilization of G-quadruplex *in-vivo* can prove to be beneficial as they may be developed as promising anti-cancer drugs since, G-quadruplexes play important roles in the repression of c-Myc [110, 111]. For neuroendocrine carcinomas (NCT00780663), the only one small-molecule compound CX-3543 (Quarfloxin) **Fig. 9** has entered phase II clinical trials. This compound is specific, and its selective interaction between the Myc G-quadruplexes and with the target sites between planes in the π-π patterns *in-vitro* the results have been based on the determination through NMR (nuclear magnetic resonance), PCR-stop, and MSi (molecular simulation studies).

Initially, CX-3543 was selected because its basic function was to disrupt nucleolin/G-quadruplex complexes in the nucleolus; thus, its activity was of a binder of Myc G-quadruplex, but later, its course of action in interaction studies with the biosynthesis of ribosomal RNA in cancer cells was deciphered, and induction of apoptosis in cancer cells was also observed [112, 113].

Perylene Derivatives: As shown in **Fig. 5**, a representative of the Perylene compound derivatives namely N,N'-bis(2-(1-piperidino)ethyl)-3, 4, 9, 10-perylenetetracarboxylic acid diimide (PIPER) is depicted, and it has been well suggested that the interaction between the Perylene derivatives and G-quadruplexes is strong [114]. End stacking interaction with G-tetrads occurs as the induction, and further, the formation of G-quadruplexes takes place from the duplex of c-Myc, i.*e.*, *via* Pu27 mer sequence [115, 116]. The binding affinity of Perylenes and their specificity and selectivity in bonding with the G-quadruplexes depends on the crucial aspect of the structure of the side chains. The fact was established as a number of perylene derivatives **Fig. 9** having linear or cyclic amines in the side chains were synthesized by Pivetta and colleagues [114]. We need to enhance the studies related to the development of novel compounds which have the basis of understanding the relationship of bonding with higher affinities and structure-specificity relationship, which can be fruitful for further synthetic studies.

Cationic Porphyrins: Based on microcalorimetric (ITC, isothermal titration calorimetry, and DSC, differential scanning calorimetry), spectrometric (UV-vis and CD, circular dichroism), and molecular simulation (MSi) studies, the representative of cationic porphyrins also a G quadruplex ligand called TMPyP4 has gained far more interest of the researcher's **Fig. 9** [117]. TMPyP7 combining abilities with the G-quadruplexes of Pu27 has shown to be substantial as it is able to down-regulate the expression level of c-Myc [110]. A drastic transformation is seen of the G-quadruplex structure when incubated with the TMPyP4; the parallel type form changes to the hybrid structure involving the parallel and anti-parallel type coordinated structure having a 1:4 ratio at a saturated condition [118]. The promotion of the formation of G-quadruplexes can be noticed when TMPyP4 uses its capabilities to interact with some G-rich sequences and stabilize i-motif structures [118, 119]. However, due to poor selectivity of the TMPyP4 the usage of the compound directly as a therapeutic agent itself is difficult to employ but has shown to prove its mantle as a leading and promising agent compound for the synthesis of novel anti-cancer therapy targeting G-quadruplexes [120].

Soon enough, a new sensation named as Se2SAP, a novel derivative, **Fig. 9**, having a porphyrin ring core was designed and synthesized by Hurley and colleagues [121]. The derivative Se2SAP gained importance because it had a higher affinity to the G-quadruplexes of c-Myc and could bind effectively to other G-quadruplexes as well as with the double-stranded DNA compared to that of TMPyP4 binding. Based on the experimental studies, it was also predicted that the parallel orientation of the c-Myc Pu27 G-quadruplex could

be converted into a hybrid G-quadruplex consisting of parallel and anti-parallel structures by the Se2SAP. Therefore, based on the conclusive experiments, it would be right to say that the first compound to hopefully identify the different conformations of G-quadruplexes is Se2SAP, and the additional bonus points are scored by the derivative as it has lower photosensitivity and has shown to be less toxic [38].

Quindolines: The sequence of c-Myc promoter Pu27 can be induced to form and stabilize the G-quadruplex structures by quindolines, and this very fact was discovered by the researcher's Gu and colleagues, thus, they after understanding the gravity of the aspect designed and synthesized a series of quindolines [122]. The growth of the tumor cells can be inhibited by modulating the repression curve of the transcription of c-Myc in HepG2 cells. Hence, these derivatives have higher affinity to G-quadruplexes than to other DNA structures and as stated previously, the side chains play as a crucial attribute in determining the binding affinity and selectivity. The primary aspect in the determination of the type of interaction is dependent on the π-π docking, and the tertiary amine in the side chains of the Quindoline derivatives **Fig. 9** such as SYUIQ-05, are more likely to interact with c-Myc G-quadruplexes than telomere G-quadruplexes [123].

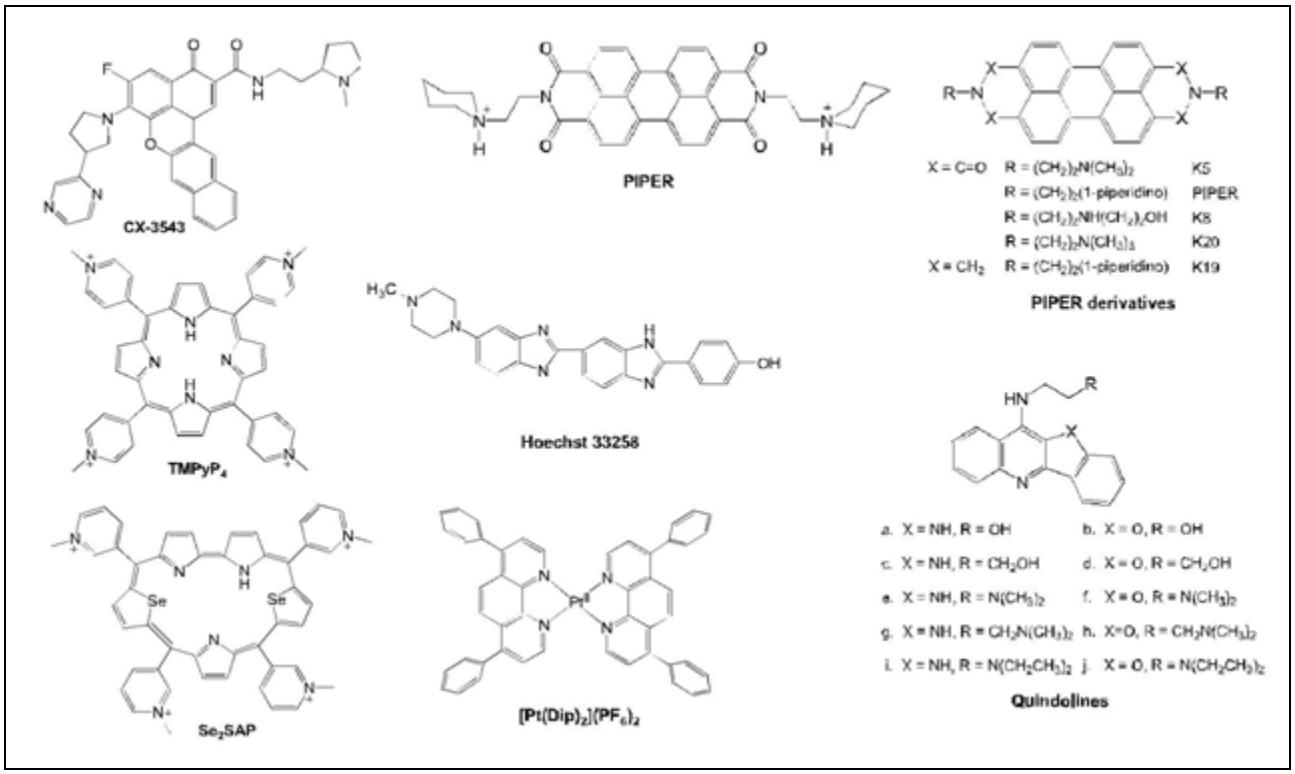

FIG. 9: SOME SMALL-MOLECULES THAT BIND c-Myc PROMOTER G-QUADRUPLEXES

Hoechst 33258: The conversion of Pu27 to the G-quadruplex is demonstrated when the synthetic dye Hoechst 33258 **Fig. 9** interact with the AAGT loop of G-quadruplexes of Pu27 [115].

Alkaloids: A ligand compound name telomestatin has been garnering a keen interest as it has shown to be one of the possibly most potent G-quadruplex ligand [126]. As from our ancestors we have heard that using natural products are beneficial and have proved to be life-saving in adverse situations moreover, their long term usage does not harm the system as well, this fact has been explored and brought into considerations for term perspective, as the natural products are a reservoir or a source of compounds with therapeutic activity and low toxicity and they can lead us in the development of tumor-selective therapies [124, 125]. Thus, exploring and targeting the natural products for pre-existing small molecules can be done diligently and further, be used for the construction of a library for screening new anti-cancer targeted drugs [127]. This very fact was understood by Ji and coworkers, and they examined the interaction of a number of natural alkaloids **Fig. 10,** and their studies showed how interaction with G-quadruplexes formed by c-

Myc Pu27 was successful. Nowadays, a series of compounds of traditional Chinese medicine (TCM), along with the variety of natural alkaloids, can interact with DNA to form complexes [128].

The representational view in **Fig. 10** shows that alkaloida such as sanguinarine (San), berberine (Beb), palmatine (Pal) and tetrahydropalmatine (Tep) can induce the formation as well as stabilize G-quadruplexes. The enhancement of the interaction of these compounds with the G-quadruplexes is done *via* the expansion of the conjugate system, the alkaloids except for Tep, the rest of these contain unsaturated ring C and positively charged centers N+ that help in the process. The alkaloid San has the highest ability to stabilize the G-quadruplex structure this was shown by the studies conducted by Ji *et al*., the study also gave emphasis on the norm that cell growth inhibition and that the interaction is mediated by external stacking or intercalation whereas, the other alkaloids with similar structures in **Fig. 10** also have comparable ability to stabilize G-quadruplexes. Further, the experiments elucidating the modified structure of Beb were used, which had 9-substituted derivative of Beb with an alkyl side chain carrying a terminal amino group which was synthesized by Huang and colleagues, and it showed higher binding affinity to G-quadruplexes than the original compound [129, 130, 131]. Similarly, another alkaloid, namely quinolino-benzo dihydro-isoquindolium (QBDI) was also transformed by modifying the 9-substituted Beb and synthesized efficiently. The interaction of these Beb and QBDI derivatives with the c-Myc G-quadruplex is observed to be with high selectivity [132].

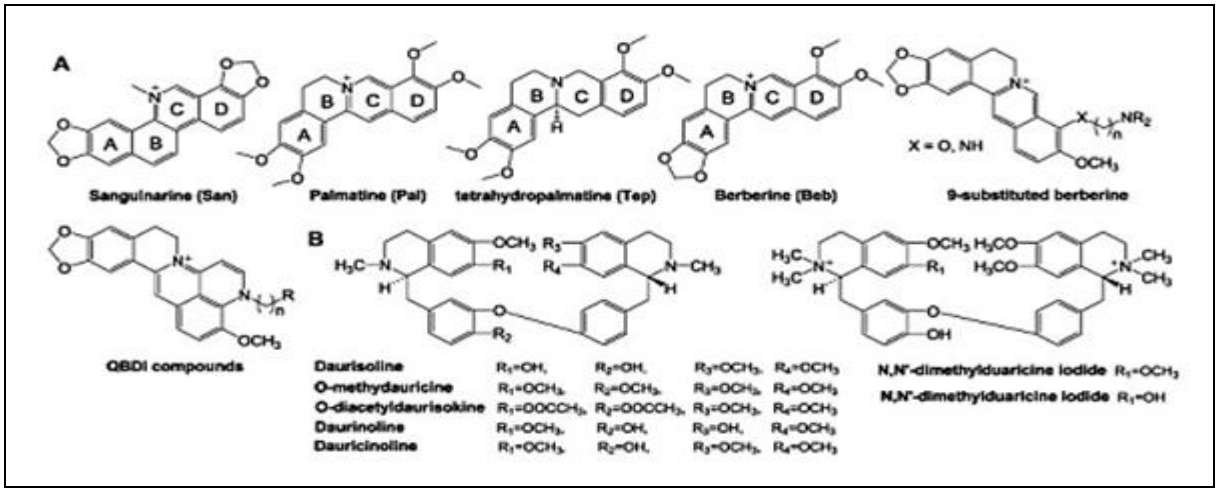

FIG. 10: STRUCTURES OF SOME NATURAL ALKALOIDS. (A) Structures of four natural alkaloids with similar structures: sanguinarine (San), palmatine (Pal), berberine (Beb), tetrahydropalmatine (Tep) and several Beb derivatives; (B) structures of 7 alkaloids with a backbone of bis-benzltetrahydroisoquinoline.

Metal Complexes: Interaction of the G-quadruplexes with a variety of small molecules containing metal is not a new phenomenon [133, 134]. It is imperative to design metal complexes drugs that are novel, well understood, and tolerated for developing anti-cancer therapies [135, 136]. Cisplatin is one of the drugs used clinically in chemotherapy, but renal toxicity and treatment-induced resistance are some of the adverse effects which are found associated with it. As stated earlier, a number of metal complexes have the ability to interact and stabilize the G-quadruplexes [137]. One such metal complex which is able to strongly interact and serves the purpose of stabilization of G-quadruplex structure is the Platinum (II) complexes (Pt (II)) [137, 138,] although, with the duplex DNA these complexes show weak binding affinities. To enhance the selectivity, Wang and coworkers designed and synthesized a Pt (II) **Fig. 9,** which showed improved action of a selection of Pt (II) binding to G-quadruplexes over duplex DNA. Thus, the complex structure [Pt(Dip)2](PF6)2 displays a greater binding affinity to c-Myc parallel G-quadruplex rather than to the duplex DNA structure as it was proved by the experimental data [139]. Other complexes have also been evaluated for binding to G-quadruplexes in addition to the Platinum, these complexes include metals containing Zn^{+}, Ni^{+}, Cu^{+}, Mn^{2+}, Ru^{2+}, $V4^{+}$ [138].

The search of novel G-quadruplexes binders that have two basic attributes, namely, selective affinity and strong binding abilities, can aid in potentially developing anticancer therapeutics. Therefore, in this regard, various metal complexes along with the well known and traditional organic heteroaromatic compounds, have emerged and shown to be an increasingly important type of compounds gaining the interest of the research community.

The repression of transcription of c-Myc G-quadruplexes is done by the small molecule ligands such as quindolines [114], cationic porphyrins [122], platinum complexes [140], which have been discussed above and they are capable of stabilizing these quadruplexes complexes. Mostly a π-π interaction is present in the G-quadruplex ligands containing aromatic planes; they form such interaction by stacking onto the ends of G-quadruplexes [134]. But, one of the examples includes Hoechst 33258, it is one of the few ligands that bind to the G-quadruplex grooves and loops having high selectivity and can possibly interact with the different topologies of G-quadruplexes which so far have been reported [141, 142]. Later it was also proved in the subsequent studies conducted by Chen *et al*. that another structure then predicted called 3, 3'-diethyloxadicarbocyanine (DODC) can too bind with the G-quadruplex grooves [143, 144, 145].

Carbamide and its Analogues: We have seen the small molecules ligands and their specificity continuing the trail from the above-discussed compounds we have a few large conjugated aromatic planes, such as Carbamide 1 **Fig. 11**. Ma and colleagues discovered this natural product, which binds with the G-quadruplexes grooves, and it shows to act as a stabilizer of c-Myc G-quadruplexes. The experimental studies conducted by the NMR and MSi conceived that carbamide 1 could control c-Myc gene transcription. The highest binding activity to the G-quadruplexes was seen when the carbamide 1 with variable diphenyl ether units was structured and earlier, the interaction of carbamide analogs 1-5 with G-quadruplexes was demonstrated [146].

Other ligands: Alongside the exploration of small-molecule ligands, the examination of the stabilization activity of c-Myc G-quadruplexes was also determined for the prospective effect of telomerase inhibitors **Fig. 11** on the structural topologies. Effective and efficient stabilization activity was observed between the c-Myc promoter and G-quadruplex complexes with the help of a variety of telomerase inhibitors which were responsible for the stability, in addition, the affinity of compounds TMPyP4 and 12459 was also deciphered in context to the c-Myc promoter G-quadruplexes and the results thus so produced revealed two-fold higher efficiency than that to the G-quadruplexes of telomere [147].

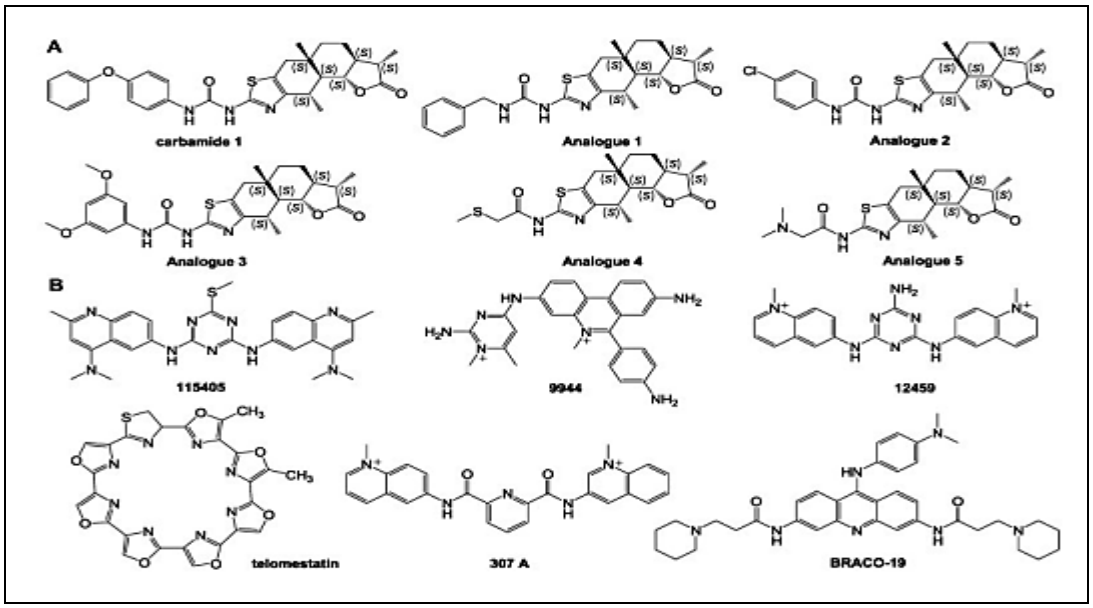

FIG. 11: CHEMICAL STRUCTURES OF A VARIETY OF G-QUADRUPLEX LIGANDS. (A) Structures of carbamide and its analogues; (B) structures of low-molecular weight ligands of telomerase promoter with affinity to the c-Myc gene promoter G-quadruplexes

TMPyP4: The structurally based drug designing has given rise to the TMPyP4, 5, 10, 15, 20-tetra (N-methyl-4-pyridyl) porphine chloride **Fig. 12D** and as a product of an attempt, its physical properties, such as the presence of a fused planar ring system, positive charge, and appropriate size to stack with the G-tetrads is significant [148.]. In HeLa cell extracts, effective stabilization of G-quadruplexes has been noticed, and it further inhibits human telomerase [148]. An account of the fact needs to be taken that over DNA duplex, the TMPyP4 exhibit significant selectivity for quad-ruplex DNA, which is stacking externally with the G-tetrad. TMPyP4 and its close analog TMPyP2 were further studied for comparison in selectivity but TMPyP2 resulted in a poor G-quadruplex-interactive compound and showed much-reduced telomerase-inhibitory activity [150]. Further, *in-vivo* studies were taken into consideration in which the data thus, so obtained clearly depicts that TMPyP4 decreases the rate of proliferation of sea urchin embryos and traps the cells in mitosis, unlike the TMPyP2. The generated data also throws light on the anaphase bridges and displays a chromosome destabilization-mediated antiproliferative effect [149]. Recent elucidation of the mechanism of TMPyP4 has revealed that it interacts with the G-quadruplex formed in the promoter region of c-MYC gene [151]. Consequently, TMPyP4 downregulates c-MYC, and this may contribute to the observed effects on telomerase by lowering hTERT, a downstream target of c-MYC [152]. However, a major hurdle in its development as a G-quadruplex target agent is its ability to bind to duplex DNA [153], RNA, RNA–DNA hybrids [154] and triplex DNA [155]. Thus, attempts have been made to generate second-generation cationic porphyrins with high selectivity for G-quadruplexes [156].

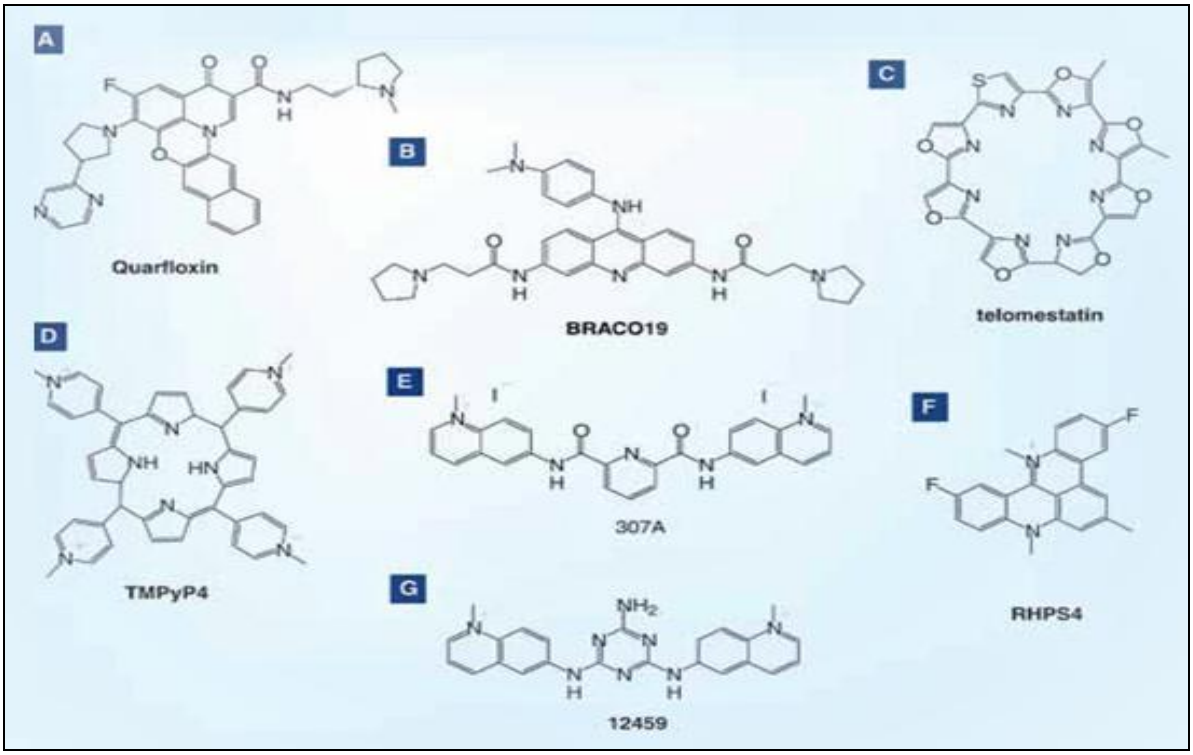

FIG. 12: G-QUADRUPLEX-INTERACTIVE SMALL-MOLECULE COMPOUNDS

307A: Based on the high selectivity of G-quadruplex-forming oligo-nucleotides, 307A was one important agent compared with mutated ones that cannot form G-quadruplexes. It is a 2, 6-pyridine-dicarboxamide derivative **Fig. 12e** [157]. The especially of this compound lies in the fact that is has shown to inhibit c-Myc gene transcription and found a ground as being useful in tumor cells and can potentially be equipotent against c-MYC and telomeric G-quadruplex-forming sequences. In telomerase positive glioma cells, when a dose-dependent sequential approach is taken, induction of apoptosis occurs within a few days; moreover, at lower concentrations, 307A and other members of this series are found to inhibit cell proliferation. SAOS2, which is a cell line involving the ALT mechanism responsible for telomere maintenance 307A had anti-proliferative effects.

As one of the outcomes of these reactions is the induced apoptosis, the chain reaction precedes leading to multiple alterations of the cell cycle, and these effects were directly proportional and related to telomere instability that involved telomere end fusion and anaphase bridge formation unlike the changes not associated with telomere shortening [158]. 307A and 360A are close analogs, and in a recent study, the role of these has been underlined substantially in the stabilization of G-quadruplexes. During *in-vitro* analysis, further extensive research has shown that incubation of oligomers with these compounds resulted in the formation of G-quadruplexes. 360A acts as a chaperone for tetra molecular complexes and not only performs the mamoth function to bind to and lock into preformed quadruplexes [159].

Proteins Involved in Transcription and Binding G-quadruplexes in Promoter Regions:

Nucleolin and Nucleophosmin: A highly expressed nucleolar phosphoprotein called the Nucleolin is seen proliferating in the cells and displays multiple roles in ribosome biogenesis[160], chromatin remodeling [161], transcription [162], G-quadruplex binding [163] and apoptosis [164]. One more interesting aspect which was witnessed is the binding of G-quadruplexes with the nucleolin-hnRNP D heterodimer structure [163, 165]. The luciferase activity in MCF10A cells measured the extent of inhibition in c-Myc promoter-driven transcription when there was an overexpression of the nucleolin phosphoprotein. Moreover, in the *in-vitro* condition, the phosphoprotein binds with high affinity and selectivity to the c-Myc G-quadruplex structure as compared to other well established and known quadruplex structures. Thus, we conclude that the formation of the c-MYC G-quadruplex structure is due to the facilitation work is done by the nucleolin, and it is also responsible for the increase in its stability. Later, studies were also conducted in the *in-vivo* conditions, and it made important revelations that the nucleolin could also bind the c-Myc promoter in such altered situations as well with sufficient efficiency [166].

Another much known multifunctional protein is Nucleophosmin, which is also considered to be playing a significant role in the pathogenesis of several human malignancies as one of the functions of this protein is in interaction with the different protein partners like, p53, p14arf, *etc*. The intrinsic unfolded C-terminal region, which contributes to the binding of c-Myc G-quadruplex motif is the key in helping and specific recognition of G-quadruplexes [168].

CONCLUSION: G-quadruplex in the secondary structures of DNA that have demonstrated themselves to be of great potential in regulating the elemental regions of biological significance, including, in human telomeres and in the promoter regions of a number of important growth-related genes. One of the most crucial facts established is that under physiological conditions, DNA G-quadruplexes can readily form in solution. The internal structure of intramolecular DNA G-quadruplexes is globular and intrinsically folded nucleic acid structures, which form a significant feature, and they are uniquely determined by the primary nucleotide sequences up to 20-30 bases in length, in an analogy to protein folding from a primary amino acid sequence. The globularly folded intramolecular G-quadruplexes obtained via NMR solution structures can be calculated from an extended single-stranded DNA, which rightfully acts as a starting model, unlike the DNA duplex.

The challenge in designing targeted drugs lies in the fact that the presence of G-quadruplexes is found in different regions of the genome, and this may pose a problem for any further development. However, the molecular structures differ from one another of intra-molecular G-quadruplexes and, therefore, in principle, different proteins and drugs can be differentially used for regulation and targeting. As discussed in this review, the G-quadruplex DNA secondary structures possess a great deal in conformational diversity, which is achieved via different folding patterns, moreover the specific loop conformations and capping structures also add up to it. Therefore, we can conclude by saying that each G-quadruplex has certain and specific folding and loop structures that approve of providing unique drug-binding pocket(s).

FUTURE PERSPECTIVE: Currently, the work is done on the structure of G4, and their recognition is preliminary, and a lot is yet to be revealed. Presently, a clear picture of how to design a chemical that can ably be used for interference

within a single gene promoter, leaving its altered expression impact without affecting the others, still remains a big task. The large planar arrangements of the external G-tetrads allows their stacking with the condensed polycyclic systems in a smooth manner. In addition, an effective yet poor specificity is encountered by the source of ligand binding and stabilization as the tetrahelical structures posses a high charge density for the whole system involved. Thus, these two main challenges are faced in dealing with the aspect; furthermore, if any alteration or mutation is done with the ligands, it can be rightly said that very little or almost no efficient interaction is left behind, which can be exploited and used for further delivery. The energetic difference of stable conformations even though G4 is highly polymorphic does not exceed1 kCal/mol.

With more accumulation of the studies and data over the period of time we can expect the emergence of a clear picture on the promoter DNA G-quadruplexes and their structures; thus, it will add up to the knowledge so far gained and exploration of more and new gene promoters which are able to form G-quadruplexes and or addition of a number of such G-quadruplexes called multiple G-quadruplexes might be discovered from a single gene promoter. New aspects would open up based on the structural studies of promoter Quadruplexes, and these will encourage the science community to explore new avenues that can provide an insight into the G-quadruplexes topology and their relationships with the DNA sequence and structure.

Due to the limited designing and synthesis, we currently have only a handful of structures which makes it even though more complicated to understand and process the binding abilities hence, respecting the need of the hour we ought to address more emphasis on structural studies which have their basis laid on drug G-quadruplex complexes, although, in a positive approach we see that the heed is being paid on the development of quadruplex specific ligands and their identification. The biological roles of G-quadruplexes and the G-quadruplex proteins which have an interactive function need to be carefully understood in order to have a better understanding of the subdue issues that can be resolved from targetting specific point source and later can also provide us with the information on G-quadruplexes that are present or formed in the RNA as well.

Developing quadruplexes interactive compounds which may be highly active is a much sort out the field from the perspective of drug development hence, the formation and synthesis of the small molecule compounds remain a critical nerve for drug designing with great emphasis being laid on selectivity and specificity towards the G-quadruplex rather than on duplex DNA structures. The inquisitive nature of the researchers towards the new quadruplex ligands is also an agenda that will cover a wide range of chemical entities. Holding the above fact in place, we can also say that highlighting the focus on the arena of telomere targetting agents will increase in the promoter G-quadruplex targeting agents as well. In the near future, we can expect the quadruplex-targeting compounds to pave their way out and enter the preclinical and clinical studies.

Based on the studies predicting the frequency of occurrence of quadruplexes in the human genome more analysis will help in building up a methodological approach which will aid us in understanding the mechanisms underlying the systems so that the clinical potential of quadruplex ligands can be achieved.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: The corresponding author Shikhar Tyagi declares that he has no conflict of interest. Shalini Rawat and Dr. Sarika Saxena declare that they have no conflict of interest.

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How to cite this article:
Tyagi S, Saxena S, Kundu N, Sharma T and Kaur S: G-quadruplex motifs in c myc promoter region and the role of various small molecule ligands/proteins in stabilizing this promoter region. Int J Pharm Sci & Res 2021; 12(1): 22-43. doi: 10.13040/IJPSR.0975-8232.12(1).22-43.