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Identification and characterisation of a G-quadruplex forming sequence in the promoter region of nuclear factor (erythroid-derived 2)-like 2 (Nrf2)

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ABSTRACT

The transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) regulates multiple antioxidants, Phase II detoxification enzymes and other cytoprotective enzymes in cells. Activation of Nrf2 is recognised as being of potential therapeutic benefit in inflammatory-diseases whereas more recently, it has become clear that the inhibition of Nrf2 may have benefit in the alleviation of resistance in some tumour types. A potential G-quadruplex forming sequence was identified in the promoter region of Nrf2, close to a number of putative transcription factor binding sites. Characterisation of the sequence 5'-d[GGGAAGGGAGCAAGGGCGGGAGGG]-3' using CD spectroscopy, imino proton NMR resonances and UV melting experiments demonstrated the formation of a parallel intramolecular G-quadruplex in the presence of K^+ ions. Incubation with 9-aminoacridine ligands induced a switch from antiparallel to parallel forms. The presence of a G-quadruplex forming sequence in the promoter region of Nrf2 suggests an approach to targeting the production of the protein through stabilisation of the structure, thereby avoiding resistance to antitumour drugs.

1. Introduction

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a member of the family of molecules known as Cap'N'collar proteins [1]. It is a transcription factor that is rapidly gaining interest as a therapeutic target due to its role as a central mediator of detoxification mechanisms within the cell [2]. Activation of Nrf2 leads to the production of antioxidant proteins, detoxification enzymes, drug efflux pumps and other cytoprotective proteins. This can have beneficial effects in inflammation and, as such, Nrf2 is gaining prominence as a target for the development of anti-inflammatory therapeutics in diseases such as chronic obstructive pulmonary disease and sepsis [3]. Under normal conditions, Nrf2 is closely controlled by interaction with a protein called Kelch-like ECH-associated protein 1 (Keap1) in the cytosol [4]. Keap1 binds to Nrf2 and induces ubiquitination and degradation. In response to oxidative stimuli, the Keap1/Nrf2 interaction is perturbed and Nrf2 accumulates in the nucleus. Here, it binds as a heterodimer to the small Maf proteins and acts as a transcription factor through binding to the antioxidant response element (ARE) on DNA [5]. This in turn leads to the expression of proteins such as heme-oxygenase 1 (HO-1) and NAD(P)H:quinone oxidoreductase I (NQO1) which act to promote the resolution of the inflammatory response [6].

To date, studies have focused on activating Nrf2 in order to generate an anti-inflammatory response. Bardoxolone-methyl is a triterpenoid that advanced into Phase III clinical trials in the treatment of type II diabetics with chronic kidney disease before being withdrawn due to unexpected toxicity [7]. It is believed to exert its effects through the inhibition of the Nrf2/Keap1 interaction via formation of a covalent adduct. Several natural and designed molecules work through a similar approach, modifying the Cys151 of the human Keap1 protein [8]. A number of reports have also been disclosed of peptides [9] and small molecules [10] that activate Nrf2 through non-covalent interaction in which the DEETGE site of the Kelch domain of Keap1 is blocked. We have also shown that cell penetrating peptide constructs using this approach can also be utilised in a cellular context and have anti-inflammatory effects [11].

While the activation of Nrf2 has potential benefits in the treatment of anti-inflammatory diseases, the inhibition of the same transcription factor may also have potential in the treatment of cancer. Several studies have shown that loss of Keap1 function leading to constitutive activation of Nrf2 generates a phenotype

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in several tumour cell lines that is resistant to chemotherapy through the activation of downstream proteins involved in the antioxidant response [12] Studies of the inhibition of Nrf2, other than through siRNA knockdown, have been limited and this may be due to the nature of the target. Activation through disruption of a protein–protein interaction (PPI), while difficult, is relatively tractable with examples of PPI inhibitors in the clinic. Alternative targets include inhibition of the degradation pathway [10]. In order to inhibit Nrf2, targets are less clear cut. One approach is to target the DNA binding sequence and there is evidence that other transcription factors may be inhibited using this approach [13]. Alternatively, inhibition of the PPI between Nrf2 and its binding partner Maf protein is also a possibility. In this paper, we disclose an alternative target – a G-quadruplex forming sequence within the promoter region of the Nrf2 gene, close to putative transcription factor binding sequences.

Higher order DNA sequences have become prominent in research since the discovery that formation of a G-quadruplex in the human telomere sequence could lead to inhibition of the enzyme telomerase, at least in *in vitro* assays [14]. The presence of putative G-quadruplex forming sequences within the promoter regions of oncogenes [15] has led to investigations of these sequences as both regulatory regions and targets for drug discovery [16]. This has been pursued most prominently with the targeting of the c-myc oncogene, although molecules that bind this sequence *in vitro* may not be working through the same mechanism *in vivo* [17]. Other higher order structures, such as the i-motif, [18] three [19] and four way junctions [20], may also have potential as drug targets, but the G-quadruplex has been the most studied of these, although selectivity against duplex DNA remains a problem.

In this study, we describe the identification and characterisation of a G-quadruplex forming sequence within the promoter region of the transcription factor Nrf2. This sequence, which is close to putative transcription factor binding sites within the promoter, has potential as a target for the down-regulation of Nrf2 in tumours where it is overexpressed or where Keap1 is mutated and inactive.

2. Materials and methods

2.1. Materials

Oligonucleotides were purchased from Eurogentec and were reverse phase HPLC purified. The ODN sequence used for Nrf2 was 5′-d[GGGAAGGGAGCAAGGGCGGGAGGG]-3′. Solid DNA samples were initially dissolved as a stock solution in MilliQ water and further dilutions were carried out in the appropriate buffer. The DNA samples were annealed by heating at 95 °C for 5 min then cooled to room temperature. Ligand **1** was previously prepared as described [21]. Final analysis and manipulation of the data was carried out using GraphPad Prism version 5.0.

2.2. UV

All UV experiments were performed on a Carey 4000 UV/Vis spectrophotometer. The thermal melting curves were obtained by monitoring the absorbance at 295 nm. Samples were prepared to final oligonucleotide concentrations of 1.25–10 μM in 10 mM sodium cacodylate (pH 7.00) containing 0–100 mM of KCl, NaCl or LiCl. They were then transferred to a masked quartz cuvette (1 cm path length), covered with a layer of silicon oil and stoppered to reduce evaporation of the sample. Samples were held at 20 °C for 5 min then heated to 90 °C twice at a rate of 0.25 °C/min, each with a 5 min hold at 20 °C and 90 °C and data was recorded every 0.1 °C during both melting and annealing. All experiments were performed at least twice and the equilibrium T_ms were determined using first derivative methods.

UV thermal difference spectra were obtained by carrying out a wavelength scan from 320 to 220 nm at 80 °C and 20 °C and plotting the difference in absorbance at each wavelength. All experiments were performed at least twice.

2.3. CD

Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter using a 1 mm path length quartz cuvette. Nrf2 ODN was diluted in a buffer containing sodium cacodylate (10 mM, pH 7.00) and additional cations (100 mM LiCl, NaCl or KCl as detailed) to achieve a total volume of 200 μL. The scans were performed at 20 °C, over a wavelength range of 220–320 nm with a scanning speed of 200 nm/min, a response time of 1 s, 0.5 nm pitch and 2 nm bandwidth. A blank sample containing only buffer was treated in the same manner and subtracted from the collected data. The CD spectra represent an average of three scans and are zero corrected at 320 nm.

2.4. NMR

^{1}H NMR spectra of the Nrf2 ODN were recorded at 298 K, using a 800 MHz Bruker Avance III spectrometer equipped with a triple resonance z-gradient probe. In order to minimise saturation of labile proton signals, jump return and watergate solvent suppression pulse sequences were used. [22] Watergate was implemented using the binomial 3-τ-9-τ-19-τ-19-τ-9-τ-3 composite pulse element with the delay time τ adjusted to 46–62 μs in order to produce a maximum of the excitation profile $1/(2\tau)$ Hz off resonance in the imino proton region of the spectrum. Samples were prepared to a final oligonucleotide concentration of 100 μM in 10 mM sodium cacodylate buffer (pH 7.00) containing 100 mM KCl and thermally annealed prior to use, 10% D_2O was then added just before commencing NMR experiments.

2.5. Fluorescence

Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer using a 1 cm path length quartz cuvette. A stock solution of **1** in water was diluted in a buffer containing sodium cacodylate (10 mM, pH 7.00) and KCl (100 mM) to achieve a total volume of 400 μL. Nrf2 ODN (40 μM) was dissolved in a buffer containing sodium cacodylate (10 mM, pH 7.00) and KCl (100 mM) and annealed. The fluorescence emission spectrum of **1** in buffer containing 100 mM KCl and 10 mM sodium cacodylate at pH 7.00 with excitation at 400 nm gives rise to a λ_{em} at 460 nm; emission spectra were recorded over a wavelength range of 410–600 nm at 20 °C. Aliquots of pre-annealed Nrf2 ODN (1 μL) were added to the solution of **1** in steps and a spectrum scanned immediately, leading to quenching of the fluorescence intensity at 460 nm in a concentration dependent manner. Portions of Nrf2 ODN were added until no further changes in fluorescence intensity were observed. The relative fluorescence $(1-(F/F_0))$ was plotted against concentration of DNA to generate a hyperbolic binding curve; we fitted this to a two site binding model (Eq. (1)) where θ is the fraction of folded DNA, K_1 and K_2 are the association constants for **1** with the Nrf2 ODN and [L] is the concentration of Nrf2 ODN added

$$\theta = \frac{k_1[\mathrm{L}] \times 2k_1k_2[\mathrm{L}]^2}{1 + k_1[\mathrm{L}] \times k_1k_2[\mathrm{L}]^2} \quad (1)$$

3. Results and discussion

The sequence of the Nrf2 promoter region was screened using the Quadparser program, which identifies putative G-quadruplex forming sequences [23]. Sequences that fold into the higher order structure generally contain a series of GGG triplets, a minimum of four, separated by short sequences that form the loops around the stacked guanine structure. Within the Nrf2 promoter region one sequence, which was at −606 from the transcription start site, was identified as a potential quadruplex forming sequence. The sequence, 5′-GGG-AAG-GGA-GCA-AGG-GCG-GGA-GGG-3′, is close to putative transcription factor binding sites in the Nrf2 promoter [24], which suggests that it has potential in the development of small molecules that stabilise the sequence in the higher order form, disrupting the duplex and preventing transcription factor binding and subsequent transcription of the Nrf2 gene. The sequence contains five G-triplets, only four of which could be involved in quadruplex formation of one structure, suggesting that multiple higher order structures are possible. It also contains two single base loops between the G-triplets at the 3′-end of the sequence, which has been suggested to be favourable for quadruplex formation and might suggest that one combination of G-sequences would be thermodynamically favoured [25]. There is one long loop 5′-AGCAA, which may be advantageous if the loops can be targeted to aid in selectivity for this sequence over other G-quadruplex sequences as well as duplex DNA.

CD spectroscopy can provide information with regard to the conformation of a G-quadruplex structure and subsequent changes in population that may occur on the addition of a ligand. Parallel quadruplexes exhibit a strong positive band at 263 nm and a negative band at 240 nm, whereas antiparallel structures show a negative band at 260 nm and a positive band at 295 nm [25]. Sequences that form both conformations or a "mixed-type" hybrid of both structures give rise to positive bands at both 263 and 295 nm. In buffer containing 100 mM KCl and 10 mM Na cacodylate at pH 7.00, the CD spectrum of Nrf2 shows a strong positive maximum at 263 nm and very little signal at 295, suggesting that a parallel conformation of a G-quadruplex is the predominant form under these conditions (Fig. 1A). Spectra for the Nrf2 sequence in 100 mM NaCl or LiCl also maintain a positive band at 263 nm but of a lower intensity to that found with potassium ions, consistent with the typical cation preference for G-quadruplexes ($K^+ > Na^+ > Li^+$) (Fig. 1A) [25,26]. In the absence of additional salt, the main band at 263 nm is absent, with the main positive band at 255 nm indicative of DNA in a random coil conformation. Interestingly, the spectrum in 100 mM NaCl also contains a positive band at 293 nm, suggesting that antiparallel or mixed-type conformations could occur under these conditions.

NMR spectroscopy has been widely used to verify the existence of G-quadruplex structure in nucleic acids [26,27]. Characteristic resonances between 10.5 and 12.5 ppm in a ^{1}H NMR spectrum are assigned to the imino-protons of guanines involved in tetrad formation, which form hydrogen bonds with the O6 of adjacent guanines. The ^{1}H NMR spectrum of Nrf2 in buffer containing 90% H_2O and 10% D_2O (Fig. 1B) demonstrates a broad envelope between 10.5 and 12.5 ppm, indicative of imino proton resonances associated with G-quadruplex tetrads. Whilst a single G-quadruplex structure can show sharp imino proton resonances, the spectrum depicted in Fig. 3 is indicative of more than one G-quadruplex conformation inter-converting [27] at a rate comparable to the NMR time scale. This is consistent with the sequence of Nrf2, which has five rather than four sets of GGG triplet repeats, allowing formation of more than one conformation.

DNA structures absorb UV light differently when folded and unfolded and taking the difference between these spectra gives an indicative spectrum which can be used to characterise different DNA secondary structures [28]. The presence of intramolecular G-quadruplex structures in a sample can be inferred by the shape of the UV difference spectra. The difference spectrum obtained for Nrf2 exhibits two positive maxima at 244 and 273 nm, a shoulder at 255 nm, and a negative minimum at 295 nm (Fig. 1C), which is comparable to thermal difference spectra of other known G-quadruplexes. The thermal stability of G-quadruplex secondary structures can be determined by UV melting experiments at 295 nm, whereby melting is coupled with a hypochromic shift, as indicated in the thermal difference spectrum. The melting transition temperature (T_m) is indicative of the stability of the folded G-quadruplex and information about the nature of the structure (inter- or intramolecular) can be deduced from experiments where strand concentration is altered. The melting and cooling curves for Nrf2, in buffer containing 100 mM KCl and 10 mM sodium cacodylate at pH 7.00, show a single transition with a T_m of 69 ± 1 °C (Fig. 2). UV melting analyses were also performed using a range of oligonucleotide concentrations between 1.25 and 10 μM and the melting transition temperature for Nrf2 was found to be independent of oligonucleotide (and strand) concentration, indicating intramolecular rather than intermolecular G-quadruplex formation. We performed UV melting experiments in varying concentrations of K^+ to observe the effects of K^+ concentration on the thermal stability of the Nrf2 sequence. As expected for G-quadruplex forming sequences, thermal stability increases with K^+ concentration (Fig. 2, T_m = 47, 62 and 69 °C for 10, 50 and 100 mM KCl respectively). Similarly, the structure is less stable in the presence of Na^+ counterions (T_m = 42 °C) and even less stable in Li^+, where a melting transition was not observed under the experimental conditions. This further supports the typical cation preference for G-quadruplex structures

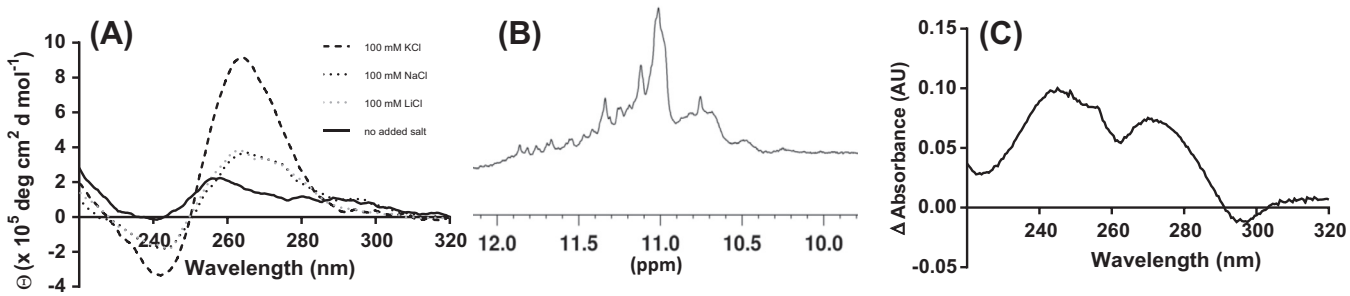

Fig. 1. Formation of a G-quadruplex by the Nrf2 promoter sequence. (A) CD spectra of Nrf2 (10 μM DNA concentration) in buffer containing sodium cacodylate (10 mM, pH 7.00, solid black line) and KCl (100 mM, black dashed line); NaCl (100 mM, black dotted line) and LiCl (100 mM, grey dotted line). (B) One Dimensional (1D) ^{1}H NMR Spectrum (at 298 K) of the Nrf2 DNA sequence (100 μM) in buffer containing KCl (100 mM), sodium cacodylate (10 mM, pH 7.00) and D_2O (10%). (C) Thermal difference spectrum (80 °C–20 °C) of Nrf2 in sodium cacodylate (10 mM, pH 7.00) and KCl (100 mM).

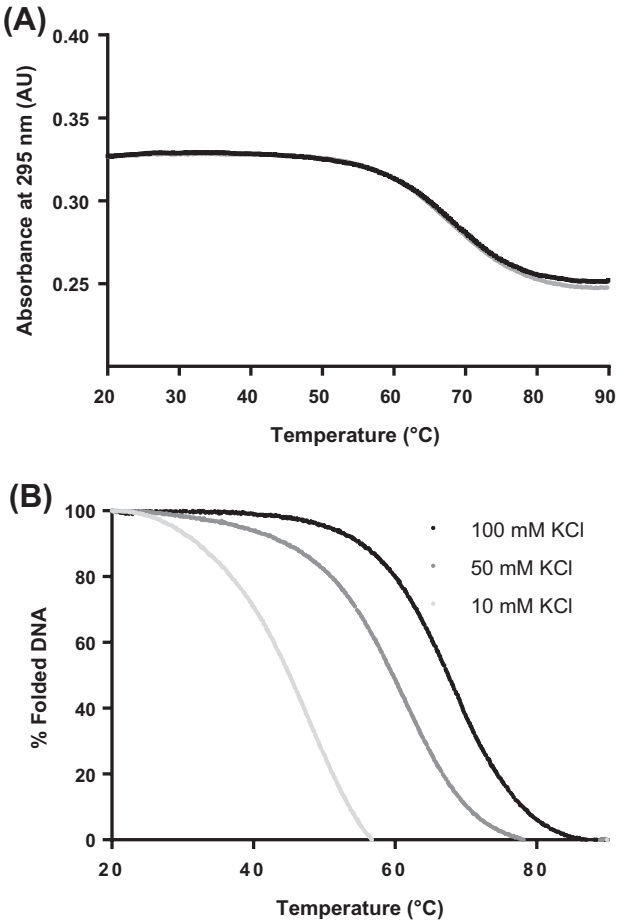

Fig. 2. The effect of K^+ ions on melting temperature of the Nrf2 promoter sequence supports formation of the G-quadruplex. (A) Heating and cooling curves for Nrf2 in the presence of 10 mM sodium cacodylate (pH 7.00) and 100 mM KCl. (B) Normalised UV meting curves for Nrf2 in the presence of 10 mM sodium cacodylate (pH 7.00) and 10 (light grey), 50 (mid grey) and 100 (black) mM KCl.

($K^+ > Na^+ > Li^+$) [26]. Taken together, the evidence from the CD, ^1H NMR, UV difference and UV melting experiments suggest that the sequence from Nrf2 forms an intramolecular G-quadruplex.

The acridine ligand family (Fig. 3) has previously been shown to have a general capacity to interact with G-quadruplex DNA sequences [21]. To further explore the potential of the Nrf2 G-quadruplex sequence as a druggable gene expression target, we utilised circular dichroism (CD) spectroscopy to study how such ligands may affect the folding topology of the DNA. The Nrf2 DNA sequence in the presence of sodium cacodylate buffer (10 mM, pH 7.00) but no additional stabilising cations such as potassium or sodium, demonstrates a slight negative peak at 240 nm and positive bands at 255, 280 and 295 nm. Addition of 10 μM of **1** to the sequence resulted in a change in the spectrum, an increase in the peak at 295 nm was observed, indicating the induction of an anti-parallel conformation of the Nrf2 sequence. Further equivalent portions were added and the peak at 295 nm was found to increase further up until 4 equivalents of ligand (40 μM). After this point, the peak at this wavelength started to decrease whereas a positive band at 263 nm was found to increase up until 120 μM (12 equivalents) at which no further changes in ellipticity were observed. The reduction in the band at 295 nm and the parallel increase in the band at 263 nm indicate switching from an anti-parallel type G-quadruplex structure to a more parallel conformation at higher ligand concentrations. Acridines are well known fluorescent molecules and we utilised this property to probe binding dissociation constants by fluorescence titrations with the Nrf2 sequence using a protocol analogous to one previously described [20]. Fluorescence titrations were performed to indicate binding affinity for **1** and the Nrf2 ODN. The saturation binding curves were fitted to an independent two-site binding model, which gave K_ds of 1.5 ± 0.01 μM and 15 ± 1.5 μM. Thus, ligand **1** shows the ability to bind and induce parallel G-quadruplex formation in the absence of any additional cations.

Nrf2 is a protein that has garnered particular interest due to its roles in inflammation and in cancer chemoprevention and there has been intense focus on developing approaches to small molecules that activate the transcription factor and its associated pathways. The fact that Nrf2 also plays a role in resistance in cancer suggests that it may be a good target for inhibition, although approaches to this are less well advanced in the literature. In this paper, we describe one potential approach through the identification of a G-quadruplex in the promoter region of the gene for Nrf2. Several other G-quadruplexes have been targeted by small molecules to generate a therapeutic effect, although none of these have yet made their way into the clinic. The recent demonstration that G-quadruplex structures could be visualised in cells [29,30], suggests that they may have value as targets to intervention and this is particularly germane for proteins such as transcription factors that do not have obvious binding sites that can be targeted for inhibition in other ways. We are currently investigating the effect of small molecules that bind to the natural parallel structure and their effects *in vivo*.

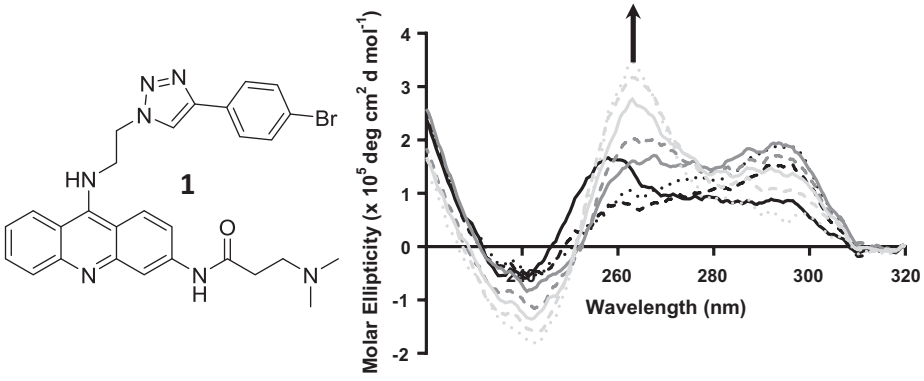

Fig. 3. Small molecule induction of the parallel G-quadruplex form of the Nrf2 promoter sequence. Acridine ligand **1** (left) and CD titration of ligand **1** (0–120 μM) into Nrf2 (10 μM) in the presence of 10 mM sodium cacodylate buffer (pH 7.00) in the absence of any additional cations.

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References

[1] P. Moi, K. Chan, I. Asunis, A. Cao, Y.W. Kan, Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 9926–9930.
[2] I.M. Copple, The Keap1–Nrf2 cell defence pathway – a promising therapeutic target?, Adv Pharmacol. 63 (2012) 43–70.
[3] A. Boutten, D. Goven, E. Artaud-Macari, J. Boczkowski, M. Bonay, Nrf2 targeting: a promising therapeutic strategy in chronic obstructive pulmonary disease, Trends Mol. Med. 17 (2011) 363–371.
[4] K. Itoh, N. Wakabayahsi, Y. Katoh, T. Ishii, K. Igarashi, J.D. Engel, M. Yamamoto, Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain, Genes Dev. 13 (1999) 76–86.
[5] K. Itoh, T. Chiba, S. Takahashi, T. Ishii, K. Igarashi, Y. Katoh, T. Oyake, N. Hayashi, K. Satoh, I. Htayama, Y. Yamamoto, Y. Nabeshima, An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements, Biochem. Biophys. Res. Commun. 263 (1997) 313–322.
[6] S.A. Rushworth, D.J. MacEwan, M.A. O'Connell, Lipopolysaccharide-induced expression of NAD(P)H:quinone oxidoreductase 1 and heme oxygenase-1 protects against excessive inflammatory responses in human monocytes, J. Immunol. 181 (2008) 6730–6737.
[7] D. De Zeeuw, T. Akizawa, P. Audhya, et al., Bardoxolone methyl in type 2 diabetes and stage 4 chronic kidney disease, N. Engl. J. Med. 369 (2013) 2492–2503.
[8] A.J. Wilson, J.K. Kerns, J.F. Callahan, C.J. Moody, Keep calm and carry on covalently, J. Med. Chem. 56 (2013) 7463–7476.
[9] R. Hancock, M. Schaap, H. Pfister, G. Wells, Peptide inhibitors of the Keap1–Nrf2 protein–protein interaction with improved binding and cellular activity, Org. Biomol. Chem. 11 (2013) 3553–3557.
[10] C. Zhuang, S. Narayanapillai, W. Zhang, Y.Y. Sham, C. Xing, Rapid identification of Keap1–Nrf2 small molecule inhibitors through structure based virtual screening and hit based substructure search, J. Med. Chem. (2014), http://dx.doi.org/10.1021/jm4017174.
[11] R. Steel, J. Cowan, E. Payerne, M.A. O'Connell, M. Searcey, Anti-inflammatory effect of a cell penetrating peptide targeting the Nrf2/Keap1 interaction, ACS Med. Chem. Lett. 3 (2012) 407–410.
[12] M.C. Jamarillo, D.D. Zhang, The emerging role of the Nrf2/Keap1 signalling pathway in cancer, Genes Dev. 27 (2013) 2179–2191.
[13] P.B. Dervan, R.M. Doss, M.A. Marques, Programmable DNA binding oligomers for control of transcription, Curr. Med. Chem. Anticancer Agents 5 (2005) 373–387.
[14] D. Sun, B. Thompson, B.E. Cathers, M. Salazar, S.M. Kerwin, J.O. Trent, T.C. Jenkins, S. Neidle, L.H. Hurley, Inhibition of human telomerase by a G-quadruplex-interactive compound, J. Med. Chem. 40 (1997) 2113–2116.
[15] J.L. Huppert, S. Balasubramanian, G-quadruplexes in promoters throughout the human genome, Nucleic Acids Res. 35 (2007) 406–413.
[16] S. Balasubramanian, L.H. Hurley, S. Neidle, Targeting G-quadruplexes in gene promoters: a novel anticancer strategy?, Nat Rev. Drug Discovery 10 (2011) 261–275.
[17] P.V. Boddupally, S. Hahn, C. Beman, B. De, T.A. Brooks, V. Gokhale, L.H. Hurley, Anticancer activity and cellular repression of c-MYC by the G-quadruplex-stabilising 11-piperazinylquindoline is not dependent on direct targeting of the G-quadruplex in the c-MYC promoter, J. Med. Chem. 55 (2012) 6076–6086.
[18] H.A. Day, C. Huguin, Z.A.E. Waller, Silver cations fold i-motif at neutral pH, Chem. Commun. 49 (2013) 7696–7698.
[19] S. Phongtongpasuk, S. Paulus, J. Schnabl, R.K. Sigel, B. Spingler, M.J. Hannon, E. Freisinger, Binding of a designed anti-cancer drug to the central cavity of an RNA three way junction, Angew Chem Int Ed Engl. 52 (2013) 11513–11516.
[20] L.A. Howell, Z.A.E. Waller, R.P. Bowater, M.A. O'Connell, M. Searcey, A small molecule that induces assembly of a four way DNA junction at low temperature, Chem. Commun. 47 (2011) 8262–8264.
[21] L.A. Howell, R.P. Bowater, M.A. O'Connell, A.P. Reszka, S. Neidle, M. Searcey, Synthesis of small molecules targeting multiple DNA structures using click chemistry, ChemMedChem 5 (2012) 792–804.
[22] M. Piotto, V. Saudek, V. Sklenar, Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions, J. Biomol. NMR 2 (1992) 661–665.
[23] H.M. Wong, O. Stegle, S. Rodgers, J.L. Huppert, A toolbox for predicting G-quadruplex formation and stability, J. Nucleic. Acids (2010) 6, http://dx.doi.org/10.4061/2010/564946. Article ID 564946.
[24] W. Miao, L. Hu, P.J. Scrivens, G. Batist, Transcriptional regulation of NF-E2 p45-related factor (NRF2) expression by the aryl hydrocarbon receptor-xenobiotic response element signaling pathway, J. Biol. Chem. 280 (2005) 20340–20348.
[25] A. Buguat, S. Balasubramanian, A sequence-independent study of the influence of short loop lengths on the stability and topology of intramolecular DNA G-quadruplexes, Biochemistry 47 (2008) 689–697.
[26] A. Wong, G. Wui, Selective binding of monovalent cations to the stacking G-quartet structure formed by guanosine 5′-monophosphate: a solid-state NMR study, J. Am. Chem. Soc. 125 (2003) 13895–13905.
[27] H. Fernando, A.P. Reszka, J. Huppert, S. Ladame, S. Rankin, A.R. Venkitaraman, S. Neidle, S. Balasubramanian, A conserved quadruplex motif located in a transcription activation site of the human c-kit oncogene, Biochemistry 45 (2006) 7854–7860.
[28] J.L. Mergny, J. Li, L. Lacroix, S. Amrane, J.B. Chaires, Thermal difference spectra: a specific signature for nucleic acid structures, Nucleic Acids Res. 33 (2005) e138.
[29] G. Biffi, D. Tannahill, J. McCafferty, S. Balasubramanian, Quantitative visualisation of DNA G-quadruplex structures in human cells, Nat. Chem. 5 (2013) 182–186.
[30] G. Biffi, M. Di Antonio, D. Tannahill, S. Balasubramanian, Visualization and selective chemical targeting of RNA G-quadruplex structures in the cytoplasm of human cells, Nat. Chem. 6 (2014) 75–80.