Research Article

Synthesis of New Poly Amide linked Heterocyclesas G-quadruplex Stabilizing Agents.

Kawkab Saour[1*], Dunya Lafta[2]
[1]Professor, Pharmaceutical Chemistry Department, Baghdad University, Iraq.
[2]Lecturer, Pharmaceutical Chemistry Department, Kufa University, Iraq.
*Corresponding author's E-mail: dunyal.mohammed@uokufa.edu.iq

ABSTRACT

In this study, we report novel compounds as polyamide compounds with aliphatic tertiary amine tail, compounds synthesized by conventional solution method employing DIC/HOBT as coupling system. The building block molecule was obtained by Chan-lam mediated oxidative coupling reaction in air; the biological activity against a panel of cancer cell line was assessed using SRB method. The results from preliminary assay indicated that introducing of imidazole heterocyclic unit instead of pyrrole in Distamycine significantly enhance the binding affinity with the quadruplex. The stabilization of non-canonical form was estimated with FRET DNA Technology using different sequences such as F21T, c-Myc, c-kit1 and c-kit2. Two member compounds (6 & 7) showed to be very selective in stabilizing one particular G-Quadruplex.

Keywords: G-quadruplex; FRET; Circular Dichroism; Cancer cell line

INTRODUCTION

An increasing interest in non-canonical nucleic acid structures has drawn the attention of the scientific community during the last few decades.[1] One such structure, the G-quadruplex, has been extensively studied, as G-quadruplexes are believed to play a significant role in cell biology such as telomere integrity and gene expression.[2] Compounds could bind and stabilize Quadruplex DNA structure have a valuable interest in anticancer drug design due to their selective inhibition of telomerase and consequence effect on cell proliferation.[3]

G-rich sequences can form G- quadruples DNA structures of stacked guanine-tetrads (G- quartet motif) formed by the coplanar arrangement of four guanines, held together by Hoogsteen bonds.[4]

Quadruplexes have been characterized in the promoter sequences of a number of proto-oncogenes and cancer-associated growth factors, notably c-Myc, c-kit, k-ras, PDGF-A and bcl-2.[5-9] The most widely studied DNA G-quadruplexes are those formed from the G-rich strand of human Telomere DNA, which has the sequence (GGTTAG)n.[10]

The human telomere protected by a protein complex called Telosome, which protects the DNA from degradation, non-homologous end joining and the activation of the DNA damage response (DDR) machinery.[11] Telomeres are shortened in somatic cells, leading to their limited life span, whereas they are stabilized in cancer cells.

The function of telomerase, which is over-expressed in > 80% of cancer cells, is to protect both physically and maintain telomere length.[12] The stabilization of G-

quadruplex structures in the single- stranded 3'telomeric DNA overhang by small-molecule ligands has been shown indirectly to inhibit telomerase and telomere maintenance in cancer cells.[13,14]

Stabilization of telomere G-quadruplexes has been proposed to disrupt telomere function and inhibit telomerase enzyme evaluation few G4-binding small molecules have proceeded to in vivo evaluation in models of human cancer.

To date only one compound, Quarfloxin has been evaluated in clinical trials[15]. The perceived lack of drug–like characteristics in many G4-binding compounds may has hindered progress to the clinic. We report here novel ligands with MW < 700 Da that could be suitable starting points for future drug discovery efforts.

We used a distamycin scaffold as a starting point and introduced biaryl building blocks in place of pyrrole rings to switch preference from duplex to quadruplex by replacing terminal heterocyclic N-pyrrol with different heterocyclic rings such as benzothiazole and imidazole so we use this scaffold as base to generate diverse molecular compounds with greater selectivity to quadruplex nucleic acid.[16]

A simple linear synthetic route was devised to prepare the targeted molecular structures, and the points of diversity were introduced using parallel chemistry.[17]

HOBT–DIC-mediated amide coupling approach and microwave-assisted Suzuki coupling were used as synthetic protocol for building up molecules.

The intermediates, as well as final compounds, were purified by catch and release method using Biotage cartridge that offer significant purity over 90% of

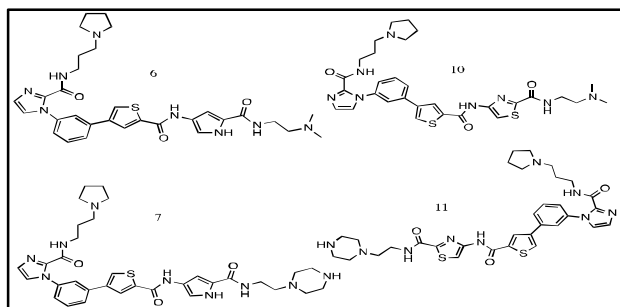compounds as this indicated by LC/MS and NMR spectra. Structures of the final compounds 6-11 described below:

Figure 1: Structure of final compounds

RESULTS AND DISCUSSION

FRET Assay

Assessment of the G-quadruplex interaction of Compounds (6–11) was initially carried out using a FRET-based melting assay[18] using fluorescence tagged Seq-3 obtained from (Eurogentec, UK, see practical section for methodology). Three different intramolecular DNA G-quadruplex sequences were used: human telomeric G-quadruplex (F21T, FAM-d (G3 [TTAG3] 3)-TAMRA) and two different c-kit promoter G-quadruplexes (c-kit1, FAM-d (G3AG3CGCT-G3AG2AG3)-TAMRA and c-kit2, FAM d (G3CG3CGCGA-G3AG4)-TAMRA)[19]. DNA duplex hairpin sequence [FAM (TA) 2GC(TA) 2T6(TA) 2GC (TA) 2-TAMRA] was used as a control.[20] Together, the melting data from both G-quadruplex and duplex sequences enabled assessment of the selectivity of the ligands for different G-quadruplex sequences. The data in Table 1 show that the poly amide compounds (6–11) exhibit significant selectivity towards G-quadruplex compared to duplex DNA, and the best molecule in the series, comp 7, has no significant action on duplex DNA and great stability for H-teloand c-kit 1 compared with other sequences the difference in molecular structures between compound 6, 7 and that of compound 10 and 11 is in the terminal heterocyclic rings which contributed to such finding results. The FRET analysis comes comparable to that of biological results and circular dichroism study.

Circular Dichroism Study

DNA samples were dissolved in Tris-HCl buffer (50 mM, pH 7.4), the samples of human telomeric G-quadruplexes also contained l00 mMKCl The CD titration of c-kit-1 quadruplexes was performed in the absence of salts. Spectra were recorded between 220 - 320 nm and 220 - 400 nm in 5 mm path length cuvettes. Results were averaged over three scans. The spectra were normalized to have zero ellipticity at 320 nm. The CD spectrum of the F21T sequence showed the presence of mixed parallel and antiparallel structures with positive peaks around 300 nm for compound 6 and around 290 for compound 7. A negative peak around 260 nm (characteristic of anti-parallel structure) was observed for both compounds 6 & 7. While, titration with c-kit1 sequence exhibit major negative peak around 240 and major positive peak

around 270. In general, titrations with the ligands (6-11) exhibited selective induction of anti-parallel G-quadruplex structures. It should be noted that for 6 and 7 a concentration dependent enhancement of the major positive peak was observed, see Figure 3 which show the CD titration of 1-6 equivalent compound 6 and 7 with F21T sequence in 100 mM HCL and Figure 4 showing CD titration of compound 6 and 7 with C-kit 1 sequence.

In vitro Growth Inhibitory Study

The short-term cell growth inhibitory activity of the biaryl ligands was evaluated using the sulforhodamine B (SRB) assay for three different cancer cell lines and a normal human lung fibroblast cell line (WI38) (Table 2).[21] The ligands generally showed greater cell growth inhibition against human colorectal carcinoma (HT29) and human lung carcinoma (A549) lines. The aim when targeting cancer cells is to achieve selectivity over normal cells. Although cell culture data can only be indicative compared to in vivo results, the data here do show that some ligands, in some cell lines, have significant selectivity. It clear from IC50 values obtained those compounds are more active against cancer cell lines as compound 6 is more active against MCF7 compared with other cancer cell lines or normal fibroblast cell, this compound gave promising results which need further extensive study for further development and sequence selectivity. Compound 7 gave more activity toward HT29 with more selectivity while compound 11 show weak selectivity toward all cell lines. Compound 7 are more selective toward HT29 cancer cell with no significant activity toward human normal cell (W138).

Experimental Section

General Method: All the reagents, starting materials as well as solvents were purchased commercially from Thermo Fischer and used without any further purification. The melting points were recorded in Sanyo Gallenkamp melting point apparatus without correction. 1H NMR and 13C NMR spectra were recorded on a Bruker Avance 400 MHz Ultra shield NMR spectrometer with TMS as internal standard and DMSO-d6 solvent unless reported, Mass spectrometric analysis was performed under ESI+ condition on a LCT Premier mass spectrophotometer. IR spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR Spectrophotometer. General scheme of synthesis used to synthesize all compounds described in Figure 5.
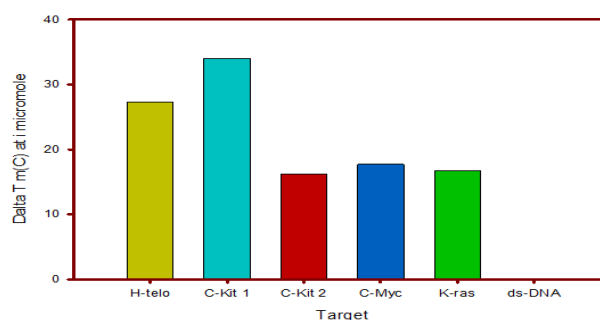

Figure 2: DNA thermal shift indicates predominance of c-kit 1 stabilization activity.
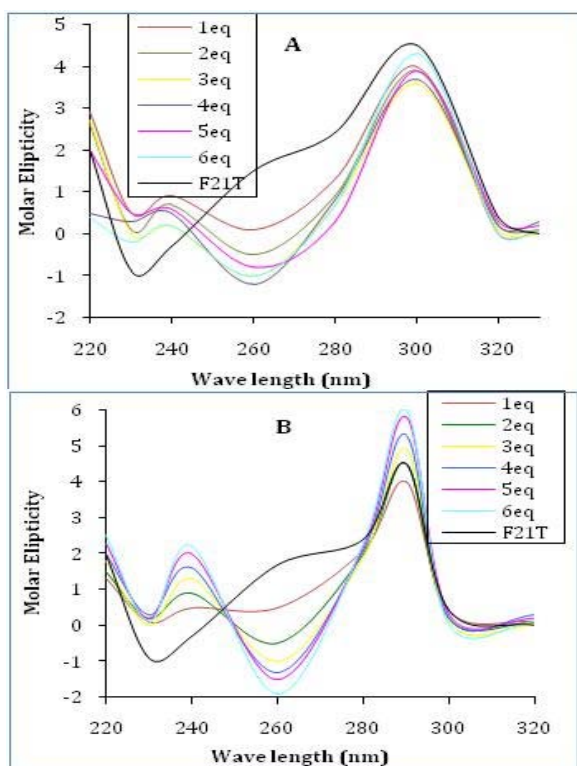
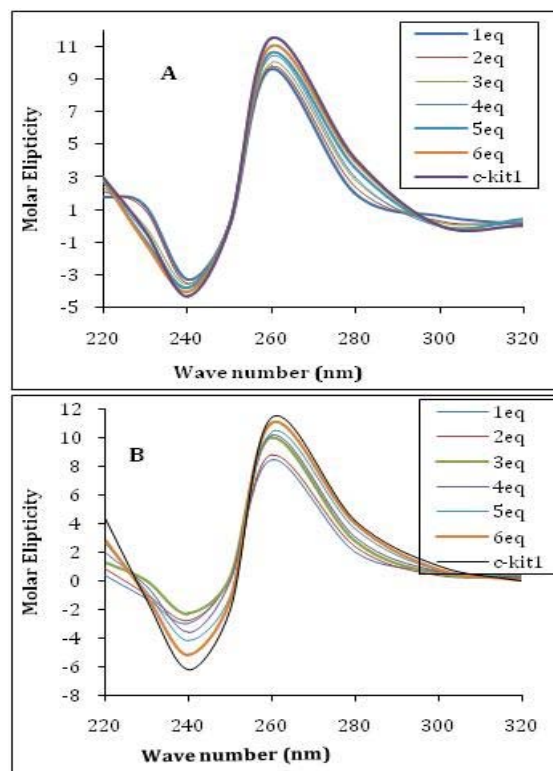**Figure 3:** CD spectra of F21T in presence of 100 mMKCl with 1-6 eq A (compound 6); B, (Compound 7).

Figure 4: CD spectra of c-kit1 with 1-6 eq. A (compound 6); B, (Compound 7).

Table 1: FRET-based DNA melting for compounds (6-11)

Code	H-telo	C-kit1	C-kit2	C-Myc	K-ras	dsDNA
6	26.3 ± 0.2	33 ± 0.3	16.2 ± 0.9	17.7 ± 0.4	16.7 ± 0.5	0.0 ± 0.2
7	25.9 ± 0.5	2.9 ± 0.3	14.9 ± 0.7	13.8 ± 0.5	20.8 ± 0.3	0.4 ± 0
10	16.7 ± 0.5	20.1 ± 0.3	6.1 ± 0.2	7.1 ± 0.4	7.1 ± 0.4	0.5 ± 0
11	16.7 ± 0.5	19.8 ± 0.4	9.3 ± 0.6	7.5 ± 0.5	16.0 ± 0.6	0.0 ± 0.2

Table 2: IC50 (μM) values of (6–11) compounds against different Cancer cell lines and a normal cell line (WI38)

Ligand Code	MCF7	A549	HT29	WI38
6	0.4	1.2	1.5	19.5
7	1.1	2.5	0.2	25.2
10	2.6	2.3	2.1	7.4
11	5.1	4.0	3.2	9.2

X= O,S,N; Y=N,C R$_1$,R$_2$ = Aliphatic tertiary amine or piperazine

(1)

Figure 5: Scheme of Synthesis

Synthesis of N-(3-(pyrrolidin-1-yl) propyl)-1H-imidazole-2-carboxamide (Compound 1)

Coupling was done by the conventional solution method. To a stirred solution of 1*H*-imidazole-2-carboxylic acid (500 mg, 4.46 mmole) in (19 ml) of DMF using flask/Green House Parallel Synthesizer vial fitted with a magnetic stirrer, then (1 gm., 7.435 mmole) HOBt and 1ml DIC was added to the suspension and stirring was continued until active ester was formed that confirmed by continue monitoring using LC/MS and not TLC. A solution of 3-(pyrrolidin-1-yl) propan-1-amine (476.59 mg, 3.717 mmole) was then added to the reaction mixture, which was then left with continuous stirring until no more 3-(pyrrolidin-1-yl) propan-1-amine was left by LC/MS monitoring. Then the reaction mixture was purified by catch and release method SCX-2 resin Biotage cartridge using 10 g sorbent capacities. The product released from cartridge using 2M NH3/Me-OH to get more than 90% purity the product further dried by using VC3000D Genevac drier to get rid of DMF. IR spectra **(cm^{-1})**: 3265 NH of amide, ν2850-2950 (C-H stretching), 1610 C=O of amide, 1630 C=N weak, ν1150 (C-O), ν1080C-N.

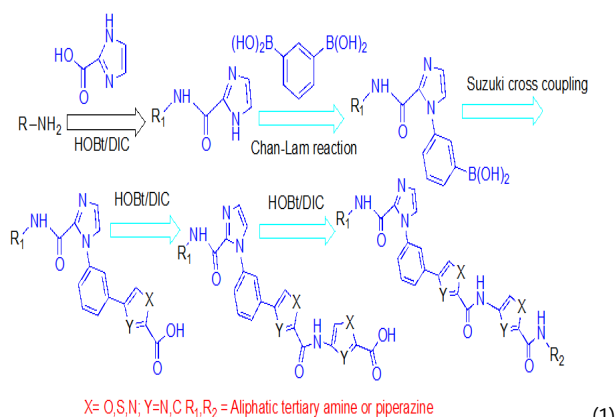### Synthesis of (3-(2-((3-(pyrrolidin-1-yl) propyl) carbamoyl)-1H-imidazol-1-yl) phenyl) boronic acid (Compound 2)

Compound 1 (500 mg, 2.25 mmole) with (816.6 mg, 4.5 mmole) boronic acid were dissolved in 100 ml DCM with sonication until completely dissolved followed by addition of (408.66 mg, 2.25 mmole copper acetate) and 587 µl of triethylamine with continuous stirring, the reaction conducted at room temperature in air until LC/MS showed the mass 343 M+ then reaction dried under vacuum and purified by catch and release method using SCX-2 resin Biotage cartridge with 5g sorbent capacities as indicated before then the product will further purified by automated flash chromatography since product difficult to obtain by ordinary column chromatography using normal flash run, then fractions dried by vacuum to get desired solid crystals with 77% yield. IR spectra **(cm^{-1})**: 3400 broad OH, ν3100 (C-H Ar. weak), ν2715-2850 (C-H), ν1400-1585(C=C Ar.), ν1000-1300(C-O), ν1080-1360 C-N.

Synthesis of 5-(3-(2-((3-(pyrrolidin-1-yl) propyl) carbamoyl)-1H-imidazol-1-yl) phenyl) thiophene-2-carboxylic acid (Compound 3)

A catalytic amount of tetrakis (triphenyl phosphine) palladium, Pd (PPh3) 4 (110.88mg, 0.51 mmole) was added to a solution of Compound 2 (661 mg, 2 mmole) with 5-bromothiophene (100 mg, 0.483mmol) in co-mixture of EtOH, toluene and water 9:3:1in the presence of K2CO3 399.4 mg in a 10-20 mL microwave vial containing a magnetic stirrer. The reaction mixture was sealed in an inert N$_2$ atmosphere and heated with microwave radiation in an EMRYS^{TM} Optimizer Microwave Station (Personal Chemistry) at 100°C for 12 minutes. After LC/MS analysis revealed completion of the reaction, the cooled reaction mixture was washed with water, extracted with EtOAc (3 x 40 mL), the filtrates combined, dried over MgSO4 and concentrated under vacuum. The resulting oil was subjected to flash chromatography (n-hexane/EtOAc 9: 1) to give 87% yield. Another method has been done at room temperature, but it gave only 40% yield. Compound 2 (661mg, 2 mmole) in Three-neck round-bottom flask fitted to reflux and magnetic stirrer with probe for temperature adjust was dissolved with 50 ml mixture of solvents (EtOH, Toluene, water) 9:3:1, followed by addition of 5-bromothiophene (100mg, 0.483 mmole) and 399.4 mg K$_2$CO$_3$ with continue stirring until starting materials dissolved partially, the other two neck closed with rubber and reaction atmosphere replaced with inert nitrogen using vacuum then Tetrakis Pd (110.88 mg) quickly added and the mixture was refluxed for several hrs. The reaction mixture monitored with LC/MS for any change. After completion of reaction the mixture dried in vacuum then purified by column chromatography using (DCM, Me-OH, NH3) as solvent system (9:0.5:1). Then it was concentrated and purified by column to provide the product as a yellow amorphous solid. IR spectra **(cm^{-1})**: 3400 broad OH, 3110 N-H stretching weak, ν3100(C-H Ar.), ν2755, 2915 (C-H), ν1000-1300(C-O),

1710 C=O carboxylic, 1600 C=O stretching of amide, ν1400-1600(C=C Ar.), ν1080-1360 C-N.

Synthesis of methyl 4-(4-(3-(2-((3-(pyrrolidin-1-yl) propyl) carbamoyl)-1H-imidazol-1-yl) phenyl) thiophene-2-carboxamido)-1H-pyrrole-2-carboxylate (Compound 4)

The acid, compound 3 (200mg, 0.471 mmole) was dissolved in DCM/DMF 10 mL in a round bottom flask/Green House Parallel Synthesizer vial fitted with a magnetic stirrer. DIC (108 µl) and HOBt (106 mg, 0.785 mmole) were added to the stirred solution at room temperature and the mixture was allowed to stirrer for hours depending on the reactivity of the acid. The amine methyl 5-amino-1*H*-pyrrole-2-carboxylate (55mg, 0.393 mmole) was added after the initial activating step, and the reaction mixture was again allowed to stirrer until TLC and LC/MS deemed the reaction complete. The reaction mixture was applied to a conditioned SCX-2 cartridge, and the coupled product was purified by "Catch and Release" method to get 85% yield. IR spectra **(cm^{-1})**: 3400 broad OH, ν3000-3100(C-H Ar.), ν2850-3000 (C-H), 1735 C=O stretching of ester, 1600 C=O stretching of amide. ν1400-1600 (C=C Ar.), ν1000-1300(C-O), ν1080-1360 C-N.

Synthesis of 4-(4-(3-(2-((3-(pyrrolidin-1-yl) propyl) carbamoyl)-1H-imidazol-1-yl) phenyl) thiophene-2-carboxamido)-1H-pyrrole-2-carboxylic acid (Compound 5)

Aqueous solution of NaOH (0.5 M) was added to a solution of the methyl ester in dioxane (1 mL for every 25 mg of ester). The reaction mixture was stirred for 6 hours at which point TLC showed completion of the reaction. Excess solvent was evaporated under vacuum, and the residue was dissolved in water. The aqueous solution was acidified with1M citric acid. The product was extracted with ethyl acetate, and the organic layer was sequentially washed with water, brine and finally dried over MgSO4. The excess solvent was removed under vacuum, and the product dried in a vacuum oven. IR spectra **(cm^{-1})**:3400-3500 broad OH carboxyl, 3200 N-H weak, ν3100(C-H Ar.), ν2750, 2900 (C-H), ν1000-1300(C-O), ν1400-1500(C=C Ar.), ν1080-1360 C-N.

Synthesis of 1-(3-(5-((5-((2-(dimethyl amino) ethyl) carbamoyl)-1H-pyrrol-3-yl) carbamoyl)thiophen-3-yl) phenyl)-N-(3-(pyrrolidin-1-yl) propyl)-1H-imidazole-2-carboxamide (Compound 6)

The acid, compound **5** (200mg, 0.532 mmole) was dissolved in DCM/DMF 10 mL in a round bottom flask/Green House Parallel Synthesizer vial fitted with a magnetic stirrer. DIC (69 µl) and HOBt (67.7 mg, 0.5 mmole) were added to the stirred solution at room temperature and the mixture was allowed to stir for hours depending on the reactivity of the acid. The amine N^1, N1-dimethylethane-1, 2-diamine (22 mg, 0.251 mmole) was added after the initial activating step and the reaction mixture was again allowed to stirrer until TLC and LC/MS deemed the reaction complete. The reaction

mixture was applied to a conditioned SCX-2 cartridge and the coupled product was purified by "Catch and Release" method described earlier with 85% yields. IR spectra **(cm⁻¹)**: 3200 N-H stretching of amide, ν3100(C-H Ar.), ν2850-2910 (C-H), ν1400-1515(C=C Ar.), ν1000-1300(C-O), ν1080-1360 C-N. ¹H NMR (500 MHz, DMSO-d₆) δ 9.24 (s, 1H), 8.50 (s, 1H), 8.38 – 8.28 (m, 1H), 8.18 – 8.04 (m, 2H), 7.99 – 7.87 (m, 1H), 7.78 – 7.63 (m, 2H), 7.64 – 7.53 (m, 2H), 7.42 (t, J = 7.5 Hz, 1H), 7.25 (s, 2H), 7.11 – 6.97 (m, 1H), 3.45 (t, J = 7.3 Hz, 2H), 3.24 (t, J = 5.0 Hz, 2H), 2.98 (t, J = 7.7 Hz, 2H), 2.73 – 2.60 (m, 4H), 2.43 (t, J = 7.3 Hz, 2H), 2.20 (s, 6H), 1.84 (dq, J = 7.1, 3.8, 3.0 Hz, 6H). ¹³C NMR (125 MHz, DMSO-d₆) δ 161.66, 160.82, 160.38, 148.08, 138.41, 137.44, 136.34, 136.05, 130.59, 130.35, 129.54, 129.52, 127.49, 123.49, 122.03, 121.30, 120.56, 119.15, 117.50, 116.20, 58.40, 54.00, 53.04, 45.69, 38.41, 37.93, 24.56, 23.40. HRMS m/z (+EI) Calculated (M+) Calculated 603.28603, found 603.2821.

Synthesis of 1-(3-(5-((5-((2-(piperazin-1-yl) ethyl) carbamoyl)-1H-pyrrol-3-yl) carbamoyl) thiophen-3-yl) phenyl)-N-(3-(pyrrolidin-1-yl) propyl)-1H-imidazole-2-carboxamide (Compound 7)

The acid, compound 5 (200mg, 0.5 mmole) was dissolved using DCM/DMF 10 mL in a round bottom flask/Green House Parallel Synthesizer vial fitted with a magnetic stirrer. The reaction mixture was cooled to -5°C using a mixture of dry ice, acetone and ice. HATU (1.3 eq.), HOAt (1.75 eq.) and DIPEA (1.5 eq.) were added to the stirred solution while maintaining the temperature at -5 °C. The amine 2-(piperazin-1-yl) Ethan amine (32.43 mg, 0.251 mmole) was added after the initial activating step and the reaction mixture was again allowed to stirrer until the reaction was deemed complete by LC/MS. IR spectra **(cm⁻¹)**: 3200 N-H stretching of amide, ν3113(C-H Ar.), ν2850-2991 (C-H), 1640 C=O of amide, ν1400-1510(C=C Ar.), ν1300(C-O), ν1080-1360 C-N. ¹H NMR (500 MHz, DMSO-d₆) δ 11.30 (s, 1H, NH Pyrol), 10.11 (s, 1H, NH amide), 8.83 (s, 1H, NH amide), 8.60 (s, 1H, NH amide), 8.39 – 8.36 (s, 1H, CH-thio), 8.35 (d, J = 2.6 Hz, 2H, CH-Imid, CH-Phen), 8.00 (d, J = 1.6 Hz, 2H, CH-thio), 7.86 (dt, J = 7.4, 2.1 Hz, 1H, CH-Phen), 7.70 (dt, J = 7.5, 2.1 Hz, 1H, CH-Phen), 7.66 (d, J = 7.5 Hz, 1H, CH Imid), 7.62 – 7.58 (m, 1H, CH-Pyrol), 7.54 (t, J = 7.5 Hz, 1H, CH-Phen), 7.07 (s, 1H, CH Pyrol), 3.37 (t, J = 7.4 Hz, 2H, N-CH2), 3.13 (t, J = 5.0 Hz, 2H, -CH2 aliph), 2.69 – 2.61 (m, 4H, N-CH2 Pyrr), 2.58 (q, J = 5.3 Hz, 4H, -CH2-NH Piper), 2.49 (t, J = 5.1 Hz, 4H, N-CH2 Piper), 2.45 (d, J = 7.4 Hz, 2H,-CH2 aliph), 2.36 (t, J = 7.7 Hz, 2H, -CH2-N aliph), 2.01 – 1.89 (m, 4H, CH2-CH2 Pyrr), 1.77 (td, J = 7.8, 3.9 Hz, 2H, -CH2-aliph), 1.72 (s, 1H, NH Piper). ¹³C NMR (125 MHz, DMSO-d₆) δ 161.66, 160.82, 160.38, 148.08, 138.41, 137.44, 136.34, 136.05, 130.59, 130.35, 129.53 (d, J = 1.9 Hz), 127.49, 123.49, 122.03, 121.30, 120.56, 119.15, 117.50, 116.20, 56.63, 54.00, 53.35, 53.04, 45.65, 37.93, 37.12, 24.56, 23.40. HRMS m/z (+EI) Calculated (M+) Calculated 644.3125 Found 644.3138.

Synthesis of methyl 4-(4-(3-(2-((3-(pyrrolidin-1-yl) propyl) carbamoyl)-1H-imidazol-1-yl) phenyl) thiophene-2-carboxamido) thiazole-2-carboxylate (compound8)

The acid, compound 3 (200mg, 0.471 mmole) was dissolved in DCM/DMF 10 mL in a round bottom flask/Green House Parallel Synthesizer vial fitted with a magnetic stirrer. DIC (108 μl) and HOBt (106 mg, 0.785 mmole) were added to the stirred solution at room temperature and the mixture was allowed to stirrer then amine methyl 4-aminothiazole-2-carboxylate (62 mg, 0.393 mmole) was added to the initial activating step and the reaction mixture was again allowed to stirrer until TLC and LC/MS deemed the reaction complete. The reaction mixture was applied to a conditioned SCX-2 cartridge, and the coupled product was purified by "Catch and Release" method described earlier to get 75% yield. IR spectra **(cm⁻¹)**: 3300 N-H of amide weak,ν3100(C-H Ar.), ν2850-3986 (C-H), 1730 C=O of ester, ν1500-1515(C=C Ar.), ν1080-1360 C-N, ν1000-1300(C-O), weak.

Synthesis of 4-(4-(3-(2-((3-(pyrrolidin-1-yl) propyl) carbamoyl)-1H-imidazol-1-yl) phenyl) thiophene-2-carboxamido) thiazole-2-carboxylic acid (compound 9)

Aqueous solution of NaOH 0.5 M was added to a solution of the methyl ester in dioxane (1 mL for every 25 mg of ester). The reaction mixture was allowed to stir for 6 hours at which point TLC showed completion of the reaction. Excess 1, 4-dioxane was evaporated under vacuum and the residue was dissolved in water. The aqueous solution was acidified with1M citric acid. The product was extracted with ethyl acetate and the organic layer was sequentially washed with water, brine and finally dried over MgSO4. The excess solvent was removed using vacuum oven to get 96% yield. IR spectra **(cm⁻¹)**: 3400-3500 broad OH, 3300 NH stretching (w), ν3100(C-H Ar.), ν1000-1300(C-O), ν2850, 2955(C-H), 1770 C=O ester, 1645 C=O of amide, ν1400-1600(C=C Ar.), ν1080-1360 C-N, ν1000-1300(C-O).

Synthesis of N-(2-(dimethyl amino) ethyl)-4-(4-(3-(2-((3-(pyrrolidin-1-yl) propyl) carbamoyl)-1H-imidazol-1-yl) phenyl) thiophene-2-carboxamido) thiazole-2-carboxamide (compound 10)

The acid, compound 9 (200mg, 0.363 mmole) was dissolved in DCM/DMF 10 mL in a round bottom flask/Green House Parallel Synthesizer vial fitted with a magnetic stirrer. DIC (83μl) and HOBt (82 mg, 0.61 mmole) were added to the stirred solution at room temperature then amine methyl 5-amino-1H-pyrrole-2-carboxylate (27 mg, 0.31 mmole) was added to the initial activating step and the reaction mixture was again allowed to stir until TLC and LC/MS deemed the reaction complete. The reaction mixture was applied to a conditioned SCX-2 cartridge and the coupled product was purified by "Catch and Release" method described earlier with 75% yield. IR spectra **(cm⁻¹)**: 3339 stretching of amide, ν3085(C-H Ar.), ν2738, 2819 (C-H) ν1665(C=O Amide), 1597 C=C of aromatic, ν1000-1300(C-O), ν1080-

1311 C-N. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (s, 1H), 9.00 (s, 1H, NH amide), 8.80 (s, 1H, NH amide), 8.35 (d, J = 7.5 Hz, 1H, NH amide), 8.26 (d, J = 2.1 Hz, 1H, CH-Imid), 8.16 (d, 1H, CH-Phen), 8.11 (s, 1H, CH-thio), 8.00 (d, J = 1.6 Hz, 1H, -CH thio), 7.83 (dt, J = 7.6, 2.1 Hz, 1H, CH-Phen), 7.62 (d, J = 3.6 Hz, 1H, CH-Imid), 7.60 (t, J = 2.0 Hz, 1H, CH-Phen), 7.48 (t, J = 7.5 Hz, 1H, CH-Phen), 6.96 (s, 1H, CH-thia), 3.37 (t, J = 7.2 Hz, 2H, -CH2 aliph), 3.13 (t, J = 7.7 Hz, 2H, -CH2 aliph), 2.71 – 2.62 (m, 4H, N-CH2 Pyrr), 2.43 (t, J = 7.3 Hz, 2H, -CH2-N aliph), 2.36 (t, J = 7.6 Hz, 2H, N-CH2 aliph), 2.20 (s, 6H, CH3-N-CH3), 2.01 – 1.91 (m, 4H, CH2-CH2 Pyrr), 1.77 (p, J = 7.6 Hz, 2H, -CH2- aliph). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.53, 162.35, 160.82, 151.36, 148.08, 139.89, 138.41, 137.44, 136.34, 136.05, 130.59, 130.35, 129.54, 123.49, 122.03, 121.30, 120.56, 119.15, 106.66, 58.40, 54.00, 53.04, 45.69, 38.41, 37.93, 24.56, 23.40.HRMS m/z (+EI) Calculated; 621.2424(M+), Found 621.381.

Synthesis of N-(2-(piperazin-1-yl) ethyl)-4-(4-(3-(2-((3 (pyrrolidin-1-yl) propyl) carbamoyl)-1H-imidazol-1 yl) phenyl) thiophene-2-carboxamido) thiazole-2-carboxamide (compound 11)

The acid, compound 9 (200mg, 0.363 mmole) was dissolved in DCM/DMF 10 mL in a round bottom flask/Green House Parallel Synthesizer vial fitted with a magnetic stirrer. DIC (83 µl) and HOBt (82 mg, 0.61 mmole) were added to the stirred solution at room temperature then 2-(piperazin-1-yl) Ethan amine (39 mg, 0.31 mmole) was added to the initial activating step and the reaction mixture was again allowed to stir until TLC and LC/MS deemed the reaction complete. The reaction mixture was applied to a conditioned SCX-2 cartridge and the coupled product was purified by "Catch and Release" method to get 83% yield. IR spectra **(cm⁻¹)**: 3300 N-H stretching of amide, N-H of piperidine within N-H of amide, ν3150(C-H Ar.), ν2950-3000 (C-H), 1655 C=O of amide, ν1556(C=C Ar.), ν1000-1300(C-O), ν1080-1360 C-N. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (s, 1H, NH amide), 9.00 (s, 1H, NH amide), 8.81 (s, 1H, NH amide), 8.34 (d, J = 7.4 Hz, 2H, CH-Thio, CH-Imid), 8.24 (d, J = 2.6 Hz, 1H, CH-Phen), 8.24 (8.00 (d, J = 1.7 Hz, 1H, CH-Thio), 7.84 (dt, J = 7.4, 2.1 Hz, 1H, CH-Phen), 7.63 (dd, J = 7.5, 4.2 Hz, 2H, CH-Phen, CH-Imid), 7.48 (t, J = 7.5 Hz, 1H, CH-Phen), 6.96 (s, 1H, CH-Thia), 3.37 (t, J = 7.4 Hz, 2H, N-CH2), 3.13 (t, J = 5.0 Hz, 2H, -CH2 aliph), 2.69 – 2.61 (m, 4H, N-CH2 Pyrr), 2.58 (q, J = 5.3 Hz, 4H, -CH2-NH Piper), 2.49 (t, J = 5.1 Hz, 4H, N-CH2 Piper), 2.45 (d, J = 7.4 Hz, 2H, -CH2 aliph), 2.36 (t, J = 7.7 Hz, 2H, -CH2-N aliph), 2.01 – 1.89 (m, 4H, CH2-CH2 Pyrr), 1.77 (td, J = 7.8, 3.9 Hz, 2H, -CH2-aliph), 1.72 (s, 1H, NH Piper). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.53, 162.35, 160.82, 151.36, 148.08, 139.89, 138.41, 137.44, 136.34, 136.05, 130.59, 130.35, 129.54, 123.49, 122.03, 121.30, 120.56, 119.15, 106.66, 56.63, 54.00, 53.35, 53.04, 45.65, 37.93, 37.12, 24.56, 23.40. HRMS m/z (+EI) Calculated 662.269(M+), Found 662.288.

FRET Method

The fluorescence tagged Seq-3 (Eurogentec, UK) stock solution in water (20 µM) was diluted to 400 nM using FRET buffer (50 mM potassium cacodylate, pH 7.4) and annealed by heating at 85°C for 5 minutes followed by cooling to room temperature over 5 hours. Ligand solution was prepared in concentrations double that required for final one. Dilutions from the initial 10 mM DMSO stock solution were performed using FRET buffer. 50 µL of annealed DNA and 50 µL of sample solution were added to each well of a 96-well plate (MJ Research, Waltham, MA) and processed in a DNA Engine Opticon. Fluorescence readings were taken at intervals of 0.5°C over the range 30-100°C, with a constant temperature maintained for 30 seconds prior to each reading. The incident radiation was 450-495 nm and detection was conducted at 515-545 nm. The raw data were imported into the program Origin (version 7.0, Origin Lab Corp.), and the graphs were smoothed using a 10-point running average and subsequently normalized. Determination of melting temperatures was performed by obtaining values at the maxima of the first derivative of the smoothed melting curves using a script. The difference between the melting temperature of each sample and that of the blank (ΔTm) was used for comparative purposes and plotted against concentration.

Sulphorhodamine B (SRB) Short-Term Cytotoxicity Assay

All cell lines were purchased from ATCC-LGC Promochem and viability was maintained in a Heraeus Hera Cell 240 incubator (37°C, 5% CO2; 75 cm plates supplied by TPP). Short-term growth inhibition was measured using a modified form of the SRB assay.

Briefly, cells were seeded (4000 cells/well MCF7 and WI38; 1000 cells/well A549; 200 cells/well HT29) into the wells of 96 well plates in the appropriate media and incubated overnight to allow the cells to attach. Subsequently, cells were exposed to freshly-made solutions of drug (dissolved in sterilized ultrapure water) at increasing concentrations between 0.1 - 50 µM quadruplicate and incubated for a further 96hr. Following this, the cells were fixed with ice-cold trichloroacetic acid (TCA) (10% w/v) for 30 min and stained with 0.4% SRB dissolved in 1% acetic acid for 15 min.

All incubations were carried out at room temperature. The IC50 values (concentration required to inhibit cell growth by 50% relative to control cells) were determined from the mean absorbance at 540nm for each drug concentration expressed as a percentage of the well absorbance in untreated control cells.

CONCLUSION

In summary, a new class of G-quadruplex ligands with high selectivity for Quadruplex DNA has been developed.

The ligands can be used as a template for generating more-potent molecules while retaining this selectivity, as well as for manipulating their selectivity to favor a

particular type of quadruplex. It is noted that there is a high potential for diversity in the terminal heterocyclic group, which should also be amenable to optimization to enhance drug-like characteristics. Future work like hybridization of G-quadruplex via the PCR-stop assay and Telomerase inhibitory activity in the telomerase TRAP-LIG assay would further enhance such concluding results.

REFERENCES

1. Schaffitzel C., Berger I., Postberg J., Hanes J., Lipps H. J, and Plu¨ck-thun A. *In vitro* generated antibodies specific for telomeric guanine-quadruplex DNA react with Stylonychialemnae macronuclei. Proc. Natl. Acad. Sci. U.S.A., 98, 2001, 8572–8577.

2. H. J. Lipps and D. Rhodes. G-quadruplex structures: *in vivo* evidence and function. Trends in Cell Biol., 19, 2009, 414–422.

3. De Cian, L. Lacroix, C. Douarre, N. Temime-Smaali, C. Trentesaux. Targeting telomeres and telomerase. Biochimie., 90, 2008, 131–155.

4. Campbell N. H., Parkinson G. N., Reszka A. P. and Neidle S. *Structural basis of DNA quadruplex recognition by an acridine drug.* Journal of the American Chemical Society, 130, 2008, 6722-6724.

5. J. L. Huppert and S. Balasubramanian. G-quadruplexes in promoters throughout the human genome. Nucleic Acids Res., 35, 2007, 406–413.

6. Phan A.T., Kuryavyi V., Burge S., Neidle S. and Patel D.J. *Structure of an unprecedented G-quadruplex scaffold in the human c-kit promoter.* Journal of the American Chemical Society, 129, 2007, 4386-4392.

7. Dai J, Dexheimer T.S, Chen D, Carver M, Ambrus A, Jones R. A, Yang D 2006 An intramolecular G-quadruplex structure with mixed parallel/antiparallel G-strands formed in the human BCL-2 promoter region in solution. *J. Am. Chem. Soc.* 128, 2006, 1096–1098.

8. Cogoi S, Quadrifoglio F, Xodo L.E, G-rich oligonucleotide inhibits the binding of a nuclear protein to the Ki-*ras* promoter and strongly reduces cell growth in human carcinoma pancreatic cells. *Biochemistry.* 43, 2004, 2512–2523.

9. S. Bashyam, V. Gokhale, H. Vankayalapati, D. Sun, A. Siddiqui-Jain, N. Streiner, K. Shin-ya, E. White, W. D. Wilson, and L. H. Hurley. Design and synthesis of an expanded porphyrin that has selectivity for the c-MYC G-quadruplex structure. J. Am. Chem. Soc., 127, 2005, 2944–2959.

10. Yang D. Z. and Okamoto K. Structural insights into G-quadruplexes: towards new anticancer drugs. Future Med. Chem, 2, 2010, 619–646.

11. D. Liu, M. S. O'Connor, J. Qin, and Z. Sonyang. Telosome, a mammalian telomere-associated complex formed by multiple telomeric proteins. J. Biol. Chem., 279, 2004, 51338–51342.

12. M. Basudeb and B. Santanu, Advances in the molecular design of potential anticancer agents via targeting of human telomeric DNA. Chem. Commun., 50, 2014, 6422—6438.

13. V. Gabelica, E. S. Baker, M. P. Teulade-Fichou, E. De Pauw, M.T. Bowers. Journal of the American Chemical Society, 129, 2007, 895-904.

14. H. J. Lipps and D. Rhodes. G-quadruplex structures: *in vivo* evidence and function. Trends in Cell Biol., 19, 2009, 414–422.

15. Balasubramanian S., Hurley L.H. and Neidle S., Targeting G-quadruplexes in gene promoters: a novel anticancer strategy? Nat. Rev. Drug Discov. 10, 2011, 261–275.

16. Michael J. B., Francisco C., Mark S., Neidle S., Synthesis of distamycin A polyamides targeting G-quadruplex DNA., Org.Biomol.Chem., 4, 2006, 3479-3488.

17. Solution-Phase Parallel Synthesis of a Multi substituted Cyclic Imidate Library Saurabh Mehta, Jesse P. Waldo, Benjamin Neuenswander, Gerald H. Lushington, and Richard C. Larock *ACS Combinatorial Science*, 15(5), 2013, 247-254.

18. Robert M. Clegg, Förster resonance energy transfer—FRET what is it, why do it, and how it's done. Laboratory Techniques in Biochemistry and Molecular Biology, Burlington: Academic Press, 33, 2009, 1-57.

19. Collie G.W. and Parkinson G.N. The application of DNA and RNA G-quadruplexes to therapeutic medicines. Chem. Soc. Rev., 40, 2011, 5867–5892.

20. Haijia Y., Xiaohui W., Manliang F., Jinsong R. and Xiaogang Q., Chiral metallosupramolecular complexes selectively recognize human tolemric G-quadruplex DNA, Nucleic acid Res., 36, 2008, 5695-57.

21. Vichai V1, Kirtikara K., Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc. 1(3), 2006, 1112-1116.