Homologous 1,3,5-triarylpyrazolines: synthesis, CH⋯π interactions guided self-assembly and effect of alkyloxy chain length on DNA binding properties†

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A series of new 2-pyrazoline derivatives (1c–12c) bearing one to twelve carbon homologous alkyloxy side chains have been synthesized in good to excellent yields via intramolecular cyclization reaction on treatment of chalcone intermediates (1a–12a) with phenyl hydrazone (1b) and characterized on the basis of their physical and spectral (IR, 1H & 13C NMR, GC-MS) data. The solid state structure of compound (2c) showed intriguing and unique 1D-supramolecular zigzag chain-like self-assembled structure, the driving force of which is only CH⋯π interactions. DNA interaction studies have also been carried out on selected compounds 1c, 3c, 5c, 6c, 9c and 12c of the series by UV-visible spectroscopy to evaluate their anticancer potential and the effect of alkyloxy chain length on DNA binding property. All the tested compounds showed strong DNA binding (10⁵–10⁶ M⁻¹ binding constants) with hyperchromic effect. A slight increase in the DNA binding strength, observed on increasing the chain length of alkyloxy groups, was attributed to their conformational arrangements, leading to the best fit conformation of 1,3,5-triaryl moiety in the minor groove of DNA structure.

1. Introduction

Among the weak non-covalent interactions, C–H⋯π interactions are considered to be the most important, in which a C–H group and a π ring act as hydrogen donor and acceptor, respectively.1−5 The energy associated with these important types of weak hydrogen bonds are significantly smaller (0.8 kcal mol⁻¹) compared to that of typical strong hydrogen-bonds between O⋯H or N⋯H (7.8 to 3.5 kcal mol⁻¹).6,7 However, these interactions have been found to be strong enough to stabilize a particular conformation of molecules for their higher-order self-assembly.6,7 These are recognized as the main non-covalent forces in protein folding that stabilize their secondary and tertiary structures8,9 in addition to their role in orienting alkyl chains toward the phenyl group of amino acid residues, binding of proteins with cofactors and carbohydrates.10 Despite tremendous progress, the study of these interactions is usually difficult because of their weak nature and their cooperative occurrence that complicate their individual study. Therefore, molecular self-assemblies driven solely by CH⋯π interactions are very important, and the molecules displaying such properties may serve as models for future studies.

Pyrazolines occupy a unique place in the family of five-membered heterocycles because of their easy synthesis and versatile pharmacological applications.11–13 Anti-tumor, anti-proliferative or anticancer potential of pyrazoline derivatives have recently been explored.14–18 Other applications of their derivatives include antiamoebic,19 antimycobacterial,20 antibacterial/antifungal,21,22 anti-inflammatory,23 antidepressant,24 neuroprotector,25 antiviral26 and anti-obesity activities.27 The pyrazoline moiety is present in a number of pharmacologically active molecules such as azolid/tandearil (anti-inflammatory), phenazone/amidopyrene/methampyrene (analgescic and antiptpyretic), anturane (uricosuric) and indoxacarb (insecticidal). Furthermore, their anti-diabetic potential has also been reviewed.28

DNA having all the genetic information/coding for cellular functions, such as cell replication and transcription, is usually a primary target for most of the anticancer drugs.29−39 Anticancer drug molecules interact with DNA through different non-covalent interactions, depending on the structure of the drug, and can modify/unwind the double stranded helical structure of DNA and destroy its normal functions, ultimately leading to cell death. The interaction of anticancer drug molecules with DNA can be categorized into three types: (i) electrostatic interaction with the anionic phosphate of DNA backbone,

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(ii) intercalation into the stacked base pairs of DNA and (iii) groove binding. Thus, DNA binding studies provide preliminary information and are of paramount importance in the discovery and development of novel anticancer agents.

Despite being a proven bioactive moiety, very little attention has been paid for studying the effect of lipophilicity on a specific bioactivity of pyrazoline derivatives. Lipophilicity has long been recognized as a meaningful parameter in determining the overall effectiveness of a candidate drug molecule. It is the most important and informative physiochemical property of a drug and plays a vital role in determining the preclinical processes of drug disposition such as absorption, distribution, metabolism, excretion and toxicity (ADMET).40–44 The control of lipophilicity within a defined optimal range not only improves the likelihood of its therapeutic success. Thus, lipophilicity determines the overall suitability of a compound to become a drug. Therefore, the role of lipophilicity in drug discovery and drug design is important and critical.

In this context and as continuation of our recent project on the synthesis and applications of pyrazolines,45–47 herein, we report the synthesis of a series of new homologous 1,3,5-triarylpyrazolines, the sole CH–π interactions guided solid state self-assembly of compound 2c and DNA binding properties to study the effect of increasing alkyloxy chain length on their DNA binding capabilities.

2. Experimental

2.1. General

All the reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as required. Thin layer chromatography (TLC) was performed using aluminium sheets coated with silica gel 60 F254 (Merck). Melting points of all the synthesized compounds have been determined in open capillary tubes using Gallenkamp apparatus (MP-D) and were uncorrected. IR spectrum in the range of 4000–400 cm⁻¹ was obtained on a Thermo Nicolet-6700 FT-IR Spectrophotometer. The 1H and 13C NMR spectra were recorded on a Bruker spectrometer at 300 MHz and 75 MHz in CDCl3, respectively, using residual solvent signals as a reference. The GC-MS spectra were recorded on Agilent 5973 inert mass selective detector in combination with Agilent 6890N gas chromatograph. The UV-Vis spectra were recorded on Shimadzu 1601 spectrophotometer.

2.2. General procedure for the synthesis of 1,3,5-triaryl-2-pyrazolines (1c–12c)

The synthesis of 1,3,5-triaryl-2-pyrazolines (1c–12c) was carried out by dissolving 4-alkyloxy chalcones [0.01 mole] in 5 mL DMF–ethanol (8 : 2) mixture containing 2–3 drops of concentrated hydrochloric acid. The reaction mixture was then heated at 60–65°C for half an hour with constant stirring before the addition of second reactant i.e. phenylhydrazine (2.16 g, 0.02 mole) (1b). After the addition of compound 1b to the reaction flask, the reaction mixture was heated to reflux for 5–6 hours. The reaction mixture was then cooled to room temperature and poured onto crushed ice. The precipitates thus obtained were filtered, washed thoroughly with distilled water and dried. To obtain highly pure compounds (1c–12c) for spectral characterization and DNA interaction studies, the obtained crude products were subjected to silica gel column chromatography using n-hexane/ethyl acetate (9:1) as the eluent.

For a better understanding of 1H NMR chemical shift values described in the experimental data, the different protons of compounds 1c–12c are labeled and shown in Fig. 1.

2.2.1. 1,3-Diphenyl-5-(4-methoxyphenyl)-2-pyrazoline (1c).

Yield 80%; pale yellow solid; m.p. 133–136°C; Rf = 0.52 (n-hexane : ethyl acetate, 9:1); UV-Vis (DMSO) λ max in nm (log ε) 362 (5.4); FT-IR: 1594 (C–N), 1175 (C–N), 1242, 1029 (Ar–O–R), 1H NMR (300 MHz, CDCl3) δ 8.14 (1H, dd, Jab = 17.1 Hz, Jax = 7.2 Hz, Hax), 3.80 (3H, s, –O–CH3), 3.83 (1H, dd, Jax = 16.8 Hz, Jfo = 12 Hz, Ho), 5.26 (1H, dd, Jax = 12.3 Hz, Jfo = 7.2 Hz, Hfo), 6.78–6.83 (1H, m, N–ArHi), 6.88 (2H, Jd = 8.7 Hz, N–ArHbi), 7.11 (2H, d, J = 7.8 Hz, ArHc/f), 7.18–7.28 (4H, m, ArHd/d), 7.34–7.44 (3H, m, ArHe/e), 7.75 (2H, d, Jf = 6.9 Hz, ArHg/g), 13C NMR (75 MHz, CDCl3) δ 43.63, 55.29, 64.00, 113.40, 114.48, 119.05, 125.73, 127.07, 128.56, 128.90, 132.82, 134.19, 134.48, 144.88, 146.72, 158.96 EIMS: m/z 328 (M+*, base peak); anal. calcd for C23H22N2O: C, 80.67; H, 6.48; N, 8.39%.

2.2.2. 1,3-Diphenyl-5-(4-ethoxyphenyl)-2-pyrazoline (2c).

Yield 84%; brown crystals; m.p. 114–116°C; Rf = 0.55 (n-hexane : ethyl acetate, 9:1); UV-Vis (DMSO) λ max in nm (log ε) 364 (5.7); FT-IR: 1596 (C–N), 1171 (C–N), 1244, 1048 (Ar–O–R), 1H NMR (300 MHz, CDCl3) δ 8.17 (1H, t, J = 6.9 Hz, O–CH2CH3), 3.15 (3H, Jf = 17.1 Hz, Jax = 7.2 Hz, Hax), 3.83 (1H, dd, Jab = 17.1 Hz, Jfo = 12.3 Hz, Ho), 4.02 (2H, q, J = 6.9 Hz, –OCH2–), 5.25 (1H, dd, Jax = 12.3 Hz, Jfo = 7.2 Hz, Hfo), 6.78–6.83 (1H, m, N–ArHi), 6.87 (2H, d, J = 8.1 Hz, N–ArHbi), 7.10 (2H, d, J = 7.8 Hz, ArHc/f), 7.18–7.28 (4H, m, ArHd/d, N–ArHg/g), 7.32–7.44 (3H, m, ArHe/e), 7.75 (2H, d, Jf = 6.9 Hz, ArHg/g), 13C NMR (75 MHz, CDCl3) δ 43.63, 55.29, 64.00, 113.38, 114.97, 119.02, 125.72, 127.05, 128.57, 128.91, 132.81, 134.19, 134.48, 144.87, 146.73, 158.33 EIMS: m/z 342 (M+*, base peak); anal. calcd for C23H22N2O: C, 80.67; H, 6.48; N, 8.25%.

2.2.3. 1,3-Diphenyl-5-(4-propoxyphenyl)-2-pyrazoline (3c).

Yield 82%; pale yellow solid; m.p. 108–109°C; Rf = 0.56 (n-hexane : ethyl acetate, 9:1); UV-Vis (DMSO) λ max in nm (log ε) 364 (5.2); FT-IR: 1595 (C–N), 1172 (C–N), 1243, 1046 (Ar–O–R),
1H NMR (300 MHz, CDCl3) δ 1.04 (3H, t, J = 7.5 Hz, –O–CH3CH2CH3), 1.81 (2H, sextet, J = 7.2 Hz, –O–CH2CH2CH3), 3.14 (1H, dd, J = 17.1 Hz, J = 7.2 Hz, H3), 3.81 (1H, dd, J = 17.1 Hz, J = 12.3 Hz, H6), 3.90 (2H, t, J = 6.6 Hz, –OCH2–), 5.25 (1H, dd, J = 16.5 Hz, J = 12.3 Hz, H2), 6.78–6.83 (1H, m, Ar–H), 6.87 (2H, d, J = 7.5 Hz, Ar-H), 7.11 (2H, d, J = 7.5 Hz, Ar–H), 7.18–7.28 (4H, m, Ar-Hd-d, Ar–Hg-g), 7.73–7.46 (3H, m, Ar–Hf-f, Ar–Hh-h); 13C NMR (75 MHz, CDCl3) δ 10.58, 22.60, 43.64, 64.03, 69.49, 113.40, 115.01, 119.02, 125.72, 127.03, 128.56, 129.80, 134.16, 134.44, 144.90, 146.72, 158.54; EIMS: m/z 356 (M+ base peak); anal. calcld for C24H24N2O4: C, 81.37; H, 7.59; N, 7.03; found: C, 81.33; H, 7.54; N, 7.21%.

2.2.7. 1,3-Diphenyl-5-(4-heptyloxyphenyl)-2-pyrazoline (7c).
Yield 83%; pale yellow solid; m.p. 78–80 °C; Rf = 0.62 (n-hexane: ethyl acetate, 9:1); UV-Vis (DMSO) λmax in nm (log ε) 365 (5.3); FT-IR: 1589 (C–N), 1169 (C–N), 1241, 1066 (Ar–O–R), 1708.7 (C=O), 1449.7, 1460.9, 1580.7 (C–N), 1328.4, 1341.4, 1344.9, 1449.0, 1476.2, 1584.5; EIMS: m/z 398 (M+ base peak); anal. calcld for C28H26N2O4: C, 80.87; H, 6.79; N, 7.86; found: C, 80.75; H, 6.70; N, 7.98%.

2.2.8. 1,3-Diphenyl-5-(4-butyloxyphenyl)-2-pyrazoline (4c).
Yield 85%; brown solid; m.p. 89–91 °C; Rf = 0.59 (n-hexane: ethyl acetate, 9:1); UV-Vis (DMSO) λmax in nm (log ε) 364 (5.2); FT-IR: 1592 (C–N), 1177 (C–N), 1242, 1067 (Ar–O–R), 1708.7 (C=O), 1449.7, 1460.9, 1580.7 (C–N), 1328.4, 1341.4, 1344.9, 1449.0, 1476.2, 1584.5; EIMS: m/z 370 (M+ base peak); anal. calcld for C22H22N2O4: C, 81.05; H, 7.07; N, 7.56; found: C, 80.90; H, 7.09; N, 7.47%.

2.2.9. 1,3-Diphenyl-5-(4-pentyl-2-phenyl-2-pyrazoline (5c).
Yield 84%; pale yellow solid; m.p. 86–89 °C; Rf = 0.62 (n-hexane: ethyl acetate 9:1); UV-Vis (DMSO) λmax in nm (log ε) 364 (5.2); FT-IR: 1593 (C–N), 1173 (C–N), 1242, 1027 (Ar–O–R), 1708.7 (C=O), 1449.7, 1460.9, 1580.7 (C–N), 1328.4, 1341.4, 1344.9, 1449.0, 1476.2, 1584.5; EIMS: m/z 384 (M+ base peak); anal. calcld for C24H24N2O5C, 81.21; H, 7.34; N, 7.29; found: C, 81.36; H, 7.39; N, 7.33%.

2.2.10. 1,3-Diphenyl-5-(4-heptyloxyphenyl)-2-pyrazoline (6c).
Yield 86%; brown solid; m.p. 87–89 °C; Rf = 0.62 (n-hexane: ethyl acetate, 9:1); UV-Vis (DMSO) λmax in nm (log ε) 364 (5.2); FT-IR: 1595 (C–N), 1172 (C–N), 1243, 1068 (Ar–O–R), 1708.7 (C=O), 1449.7, 1460.9, 1580.7 (C–N), 1328.4, 1341.4, 1344.9, 1449.0, 1476.1, 1585.6; EIMS: m/z 370 (M+ base peak); anal. calcld for C28H26N2O4: C, 81.65; H, 8.03; N, 6.57; found: C, 81.54; H, 7.91; N, 6.63%.

2.2.11. 1,3-Diphenyl-5-(4-nonyloxyphenyl)-2-pyrazoline (9c).
Yield 85%; pale yellow solid; m.p. 90–92 °C; Rf = 0.64 (n-hexane: ethyl acetate, 9:1); UV-Vis (DMSO) λmax in nm (log ε) 363 (5.3); FT-IR: 1595 (C–N), 1170 (C–N), 1241, 1014 (Ar–O–R), 1708.7 (C=O), 1449.7, 1460.9, 1580.7 (C–N), 1328.4, 1341.4, 1344.9, 1449.0, 1476.3, 1585.6; EIMS: m/z 426 (M+ base peak); anal. calcld for C32H30N2O4C, 81.65; H, 8.03; N, 6.57; found: C, 81.54; H, 7.91; N, 6.63%.
2.2.10. 1,3-Diphenyl-5-(4-decyloxyphenyl)-2-pyrazoline (10c).

Yield 81%; pale yellow solid; m.p. 90–91°C.

1H NMR (300 MHz, CDCl3) δ 0.90 (3H, t, Jax = 7.2 Hz, –O–CH2CH2–(C3)0–CH3), 1.28–1.47 (18H, m, –O–CH2–CH2–(C3)0–CH3), 3.13 (1H, dd, Jlab = 17.1 Hz, Jax = 7.5 Hz, H3), 3.83 (1H, dd, Jlab = 17.1 Hz, Jbx = 12.3 Hz, H3), 7.17–7.28 (4H, m, ArH d=d), 7.30–7.47 (3H, m, ArH f=f), 7.75 (2H, d, J = 6.9 Hz, ArHc=c), 7.80–7.87 (2H, m, ArHd=d), 7.91 (2H, d, J = 6.9 Hz, ArHe=e), 8.45 (2H, t, Jax = 7.2 Hz, Hb), 8.17 (2H, d, J = 6.9 Hz, ArHc=c).

13C NMR (75 MHz, CDCl3) δ 14.17, 22.71, 26.08, 29.30, 29.43, 29.56, 31.91, 34.76, 43.64, 64.05, 68.01, 113.40, 115.00, 119.02, 125.73, 127.03, 128.54, 128.89, 132.84, 134.14, 134.41, 144.90, 146.71, 158.56; EIMS: m/z 440 (M*, base peak); anal. calcd for C30H36N2O: C, 81.89; H, 8.42; N, 6.16; found: C, 81.83; H, 8.23; N, 6.32%.

2.2.11. 1,3-Diphenyl-5-(4-undecyloxyphenyl)-2-pyrazoline (11c).

Yield 86%; pale yellow solid; m.p. 90–92°C.

1H NMR (300 MHz, CDCl3) δ 0.90 (3H, t, Jax = 7.2 Hz, –O–CH2CH2–(C3)1–CH3), 1.28–1.47 (14H, m, –O–CH2–CH2–(C3)1–CH3), 3.31 (1H, dd, Jlab = 17.1 Hz, Jax = 7.5 Hz, H3), 3.83 (1H, dd, Jlab = 17.1 Hz, Jbx = 12.3 Hz, H3), 7.17–7.28 (4H, m, ArH d=d), 7.30–7.47 (3H, m, ArH f=f), 7.75 (2H, d, J = 6.9 Hz, ArHe=e), 8.45 (2H, t, Jax = 7.2 Hz, Hb), 8.17 (2H, d, J = 6.9 Hz, ArHc=c), 8.19 (2H, d, J = 6.9 Hz, ArHc=c).

13C NMR (75 MHz, CDCl3) δ 14.17, 22.71, 26.08, 29.30, 29.43, 29.56, 31.91, 34.76, 43.64, 64.05, 68.01, 113.40, 115.00, 119.02, 125.73, 127.03, 128.54, 128.89, 132.84, 134.14, 134.41, 144.90, 146.71, 158.56; EIMS: m/z 440 (M*, base peak); anal. calcd for C31H38N2O: C, 81.89; H, 8.42; N, 6.16; found: C, 81.83; H, 8.23; N, 6.32%.

2.2.12. 1,3-Diphenyl-5-(4-dodecyloxyphenyl)-2-pyrazoline (12c).

Yield 83%; pale yellow solid; m.p. 90–92°C.

1H NMR (300 MHz, CDCl3) δ 0.90 (3H, t, Jax = 7.2 Hz, –O–CH2CH2–(C3)2–CH3), 1.28–1.47 (18H, m, –O–CH2–CH2–(C3)2–CH3), 3.13 (1H, dd, Jlab = 17.1 Hz, Jax = 7.5 Hz, H3), 3.83 (1H, dd, Jlab = 17.1 Hz, Jbx = 12.3 Hz, H3), 7.18–7.28 (4H, m, ArH d=d), 7.30–7.48 (3H, m, ArH f=f), 7.75 (2H, d, J = 6.9 Hz, ArHe=e), 8.45 (2H, t, Jax = 7.2 Hz, Hb), 8.19 (2H, d, J = 6.9 Hz, ArHc=c).

13C NMR (75 MHz, CDCl3) δ 14.17, 22.71, 26.08, 29.30, 29.43, 29.56, 31.91, 34.76, 43.64, 64.05, 68.01, 113.40, 115.00, 119.02, 125.73, 127.03, 128.54, 128.89, 132.84, 134.14, 134.41, 144.90, 146.71, 158.56; EIMS: m/z 440 (M*, base peak); anal. calcd for C32H40N2O: C, 82.11; H, 8.77; N, 5.80; found: C, 82.01; H, 8.84; N, 6.02%.

2.3. Crystallographic data collection and structural refinement

The single crystal X-ray diffraction measurements for the compound 2c were carried out using Bruker APEX-II CCD area-detector equipped with a graphite monochromator at 296(2) K with MoKα radiations (λ = 0.71073 Å). The structure is solved by direct methods and full-matrix least-squares refinement on F2 and 236 parameters for 4323 unique intensities (Rint = 0.011). The crystal data and refinement details of compound 2c are summarized in Table 1.

2.4. DNA binding studies

Salmon sperm DNA was solubilized in double-distilled water to prepare a stock DNA solution having 5 × 10−4 M concentration, which was stored at 4°C. For measuring binding interactions, solutions of varying DNA concentrations were prepared from this stock DNA solution by dilution. The concentration of stock solution was measured by UV absorbance at 260 nm using a molar extinction coefficient of 6600 M−1 cm−1. The ratio factor value (Asample/Asolution > 1.8) suggested that the DNA was free of protein moiety. All the spectroscopic titrations were carried out by keeping the concentration of test compound constant in

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the sample cell while varying the concentration of DNA from 5 μM to 25 μM. All the samples were equilibrated at room temperature for 5 minutes prior to measurements.

3. Results and discussion

3.1. Chemistry

The compounds (1c–12c) were synthesized by reacting 4-alkyloxy chalcones (1a–12a) with two equivalents of phenyl hydrazine in DMF–ethanol solvent mixture (8:2) containing 2–3 drops of conc. HCl under reflux conditions for 5–6 hours and were purified by silica gel column chromatography using n-hexane–ethyl acetate as the mobile phase (Scheme 1). All the products were obtained as solids in 80–87% yield. The structure of all the compounds was confirmed on the basis of their FT-IR, 1H NMR, 13C NMR and EIMS data. In addition, single-crystal X-ray analysis was used to unambiguously confirm the three dimensional structure of compound 2c.

3.2. Spectral characterization of 1c–12c

In FT-IR spectroscopy of compounds (1c–12c), two absorption bands in the range of 1596–1589 cm⁻¹ and 1177–1169 cm⁻¹ were assigned to carbon–nitrogen (C=N) double bonds and carbon–nitrogen single bonds (C–N), respectively, indicating the formation of pyrazoline skeleton. In addition, two strong bands at stretching frequencies in the range of 1244–1230 cm⁻¹ and 1068–1000 cm⁻¹ were also observed, which indicates the presence of Ar–O–R groups in the synthesized compounds.

Furthermore, the methyl and methylene protons of alkyloxy groups adjacent to oxygen atom (ArO–CH₂–) were observed in the range of 3.80–4.02 ppm. The characteristic signals for methylene (–CH₂–) and methine carbon (–CH–) of the five-membered pyrazoline rings were observed in the range of 43.62–43.64 ppm and 55–64 ppm, respectively, which further support the conclusions drawn from FT-IR and 1H NMR spectroscopy. All the aromatic carbons of compounds (1c–12c) were found at their respective places in the aromatic region, ranging from 113–158 ppm.

The formation of the proposed structures of compounds (1c–12c) discussed in IR, 1H & 13C NMR spectroscopy was further confirmed by their EIMS analysis. The molecular ion peaks (M⁺) for all the compounds were observed at their respective molecular masses. Furthermore, fragmentation pattern was also in good agreement with the already reported 2-pyrazoline derivatives. The most stable fragment or base peak in compounds (1c–12c) was the molecular ion peak. The molecular mass data of all the synthesized 1,3,5-triarylpyrazolines (1c–12c) are provided in the experimental section. However, a representative compound (12c) with a proposed mass fragmentation pattern, where molecular ion peak and most stable fragment, [C₃₅H₄₂N₂O⁺], appeared at m/z 482 (Calcd 482.33) is shown in Fig. S1 (ESI†).

3.3. Solid state self-assembly

To study the packing properties of 1,3,5-triarylpyrazolines (1c–12c), good-quality single crystals of compound 2c suitable for X-ray analysis were grown in ethanol solvent by slow evaporation at

![Fig. 2 ORTEP diagram and atom labeling of 2c.](image-url)
ambient conditions and were found to have a monoclinic crystal lattice with the $P2_1/c$ space group. The ORTEP representation and atom labeling of the compound $2c$ is shown in Fig. 2. The two phenyl rings present on the 1 and 3 positions of the central pyrazoline moiety are nearly in the same plane as that of pyrazoline, making a dihedral angle of 2.97° ($N_1$–$N_2$–$C_{18}$–$C_{19}$) and 0.88° ($N_1$–$C_{11}$–$C_{12}$–$C_{13}$), respectively. The aryl ring with an alkoxy side chain substituted on the asymmetric carbon of a pyrazoline moiety is oriented in such a manner that one of its hydrogens is located almost on the top of the pyrazoline moiety at 2.803 Å distance from the centre of pyrazoline ring. The angles around the asymmetric carbon are 112.76° ($C_1$–$C_9$–$C_{10}$), 113.76° ($C_1$–$C_9$–$N_2$), 109.51° ($C_1$–$C_9$–$H_9$), 109.53° ($N_2$–$C_9$–$H_9$). In the pyrazoline moiety, the C–N, C=–N and N–N bond lengths are 1.472 Å, 1.285 Å and 1.385 Å, respectively.

The most important feature of this compound is the formation of 1D-supramolecular zigzag chains in the solid state packing, stabilized merely by CH–π interactions53 (Fig. 3). It is worth mentioning here that compounds with only one type of non-covalent interactions in their packing are either very rare or not reported at all in the literature. As shown in Fig. 3, the molecules of $2c$ are connected with each other by CH–π interactions [$C(20)$–$H(20)$···$C(17)$ 2.789 Å, $C(20)$–$H(20)$···$C(12)$ 2.883 Å], where C–H of aryl substituted at $N_2$ of the pyrazoline moiety of one molecule is interacting with the π-system of an aryl ring present at the 3-position of pyrazoline moiety of the other molecule, forming a 1D-supramolecular zigzag chain-like structure. The individual chains of this assembly are also connected with each other with CH–π interactions [$C(9)$–$H(9)$···$C(19)$ 2.630 Å, $C(9)$–$H(9)$···$C(20)$ 2.739 Å], but at this time hydrogen at the

![Fig. 3 1D-supramolecular zigzag chains of $2c$ solely mediated by CH–π interactions [C(20)–H(20)···C(17) 2.789 Å, C(20)–H(20)···C(12) 2.883 Å, C(9)–H(9)···C(19) 2.630 Å, C(9)–H(9)···C(20) 2.739 Å] viewed along (a) a-axis (b) b-axis (c) c-axis.](image-url)
asymmetric carbon of the pyrazoline moiety is interacting as donor with the π-system of an aryl ring substituted at N2 of the pyrazoline moiety of a neighboring chain. The solid state assembly of this molecule presents a perfect example of CH–π interactions-driven self-assemblies. Therefore, compound 2c can be used as a model to explore molecular self-assemblies and materials containing exceptional properties driven only by CH–π interactions. Various computational methods can be of great help to study the detailed nature of this type of weak interactions prior to any design using compound 2c as a model.

3.4. DNA binding studies

UV-visible absorption spectroscopy was used to study the interactions of the synthesized compounds (1c–12c) with DNA, which is a valuable technique for the determination of the strength and binding mode of drug–DNA interactions. In this technique, the variations in absorption intensity and wavelength are measured for the evaluation of binding parameters that reflect the corresponding changes in DNA conformation and structures after the drug binds to DNA. In compounds (1c–12c), aryl groups at 1- and 3-positions of a pyrazoline ring form the conjugated backbone and are mainly responsible for the absorption of photons, whereas the aryl group present at the 5-position of the pyrazoline is not a part of this conjugated system. Therefore, the substitution of alkyloxy group at aryl group (not part of the conjugated backbone) can be expected to have some impact on physico-chemical properties.

Compounds 1c, 3c, 5c, 6c, 9c and 12c were selected for DNA binding studies. In the electronic spectrum of all the tested compounds, an intense band at 362–365 nm was observed, which is attributed to transitions between π–π* energy levels. An increase in absorption intensity i.e. hyperchromism (29% after 5 μM, 43% after 10 μM, 53% after 15 μM, 63% after 20 μM and 65% after 25 μM addition of DNA) with no shift in \( \lambda_{\text{max}} \) was noted by the incremental addition of DNA for all the tested compounds (Fig. 4). Such a spectral characteristic is indicative of groove binding, which ultimately leads to intercalation as the dominant mode of interaction. Hyperchromism results from the denaturing (unwinding) of DNA double helical structure, whereas the contraction of DNA in the helix axis, as well as from the change in conformation in DNA leads to hypochromism (decrease in absorption intensity). The DNA binding affinity of 1,3,5-triaryl-pyrazolines was quantified according to the Benesi–Hildebrand equation.

\[
\frac{A_0 - A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \cdot \frac{1}{[\text{DNA}]K}
\]

where \( K \) is the association/binding constant and \( A_0 \) and \( A \) are the absorbances of the free and DNA bound compound, respectively. \( \varepsilon_G \) and \( \varepsilon_{H-G} \) are the molar extinction coefficients of the compound and its DNA adduct, respectively. The association constants were obtained from the intercept-to-slope ratios of \( A_0/[A - A_0] \) vs. \( 1/[\text{DNA}] \) plots. The Gibb’s free energy (\( \Delta G \)) was determined from the equation \( \Delta G = -RT\ln K \), where \( R \) is the gas constant (8.314 J K\(^{-1}\) mol\(^{-1}\)) and \( T \) is the temperature (298 K).

The binding constants and Gibb’s free energies for compounds 1c, 3c, 5c, 6c, 9c and 12c are shown in Table 2. As it is clear from the binding constant values, all the tested compounds showed strong binding with the DNA. An increase in binding strength was observed with the increase in the alkyloxy chain length, which may be attributed to its different conformational arrangements, leading to the best fit conformation of the 1,3,5-triaryl moiety in the minor groove of the DNA. Among the tested compounds, 12c was found to be the most active compound of the series with binding constant value of \( 1.17 \times 10^6 \) M\(^{-1}\). Furthermore, the interaction

<table>
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<tr>
<th>Compd no</th>
<th>R</th>
<th>Binding constant ( K ) (M(^{-1}))</th>
<th>Binding energy ( \Delta G ) (kJ mol(^{-1}))</th>
</tr>
</thead>
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<tr>
<td>1c</td>
<td>C(_3)H(_7)</td>
<td>3.08 \times 10^5</td>
<td>-31.31</td>
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<tr>
<td>3c</td>
<td>C(_3)H(_7)</td>
<td>2.63 \times 10^5</td>
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<td>1.05 \times 10^5</td>
<td>-28.64</td>
</tr>
<tr>
<td>6c</td>
<td>C(_3)H(_7)</td>
<td>7.70 \times 10^5</td>
<td>-33.58</td>
</tr>
<tr>
<td>9c</td>
<td>C(_3)H(_7)</td>
<td>1.15 \times 10^6</td>
<td>-34.57</td>
</tr>
<tr>
<td>12c</td>
<td>C(<em>{12})H(</em>{25})</td>
<td>1.17 \times 10^6</td>
<td>-35.00</td>
</tr>
</tbody>
</table>

Fig. 4 Absorption spectrum of 5c (15 μM) in the absence (a) and presence of 5 μM (b), 10 μM (c), 15 μM (d), 20 μM (e), 25 μM (f) of DNA (A). The arrow direction indicates increasing DNA concentration. Plot of \( A_0/A - A_0 \) vs. 1/[DNA] for the determination of binding constant (K) and Gibb’s free energy (\( \Delta G \)) (B).
of the compounds with DNA is a spontaneous process, as indicated by the negative values of $\Delta G$. From the results of the present study, it is quite clear that the increase in the length of the alkyloxy group significantly affects DNA binding despite having the same skeleton. Apart from this strong binding, the compounds of the present series with good lipophilic character are ideal candidates for in vitro anticancer studies against different cell lines.

4. Conclusions

In summary, we have synthesized a series of new homologous 1,3,5-triaryl-2-pyrazolines (1c–12c) with phenyl hydrazine (1b) in 80–87% yields and characterized them by their physical and spectral (IR, $^1$H & $^{13}$C NMR, GC-MS) data. Single crystal X-ray diffraction analysis of compound 2c showed 1D-supramolecular zigzag chain-like molecular self-assembly in the solid state. The specialty of this self-assembled structure is the presence of only CH–π interactions, which is not only involved in the formation of chains but also in the connection of different chains. Because molecular self-assemblies stabilized solely by one type of weak interactions are rare, the compound 2c may serve as the model to obtain the complete understanding of this non-covalent interaction. Furthermore, the interaction of six selected compounds with DNA was studied by UV-visible spectroscopy to get preliminary information regarding their anticancer potential and to determine the role of alkyloxy chain length on DNA binding property. All the tested compounds showed strong binding, and compound 12c was found to be the most active compound of the series. The difference in the binding of compounds having the same skeleton but different alkyloxy chains is attributed to their different conformational arrangements resulting in the best fit conformation of the 1,3,5-triaryl moiety in the minor groove of DNA.

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