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Synthesis and crystallographic analysis of short pyridine-based oligoamides as DNA-targeting supramolecular binders

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In this study, the synthesis of the pyridine peptides **1**–**4**, formed from amino-picolinic acid, and pyridine peptide **5**, made from coupling of a mono-protected pyridine diamine to a mono-protected pyridine dicarboxylic acid and the X-ray crystallographic structures of **1** and **5** are discussed, along with their supramolecular interactions in the solid state. The structure of these compounds showed that they possess concave appearance which can be employed to bind to nucleic acids through multiple hydrogen bonding, which would facilitate the formation of helical-based pyridine oligoamides as novel DNA-binding molecules. This was proven by carrying out DNA denaturation studies and ethidium displacement assay on **5**, but, by using MTT assays, **5** was shown to be cytotoxic against drug-resistant chronic myeloid leukaemia K562 cell-line.

Keywords: oligoamides; pyridine; DNA binders; X-ray crystallography

Introduction

The development of novel polyamide ligands, such as aromatic oligoamides (*1*, *2*), for investigating folding conformations in solution and/or self-assembly formation, is of great current interest within the field of supramolecular chemistry (*3, 4*). Such 'non-natural' polyamide-based structures, based on the use of pyrrole–imidazole polyamides and bicyclic ring structures, have also been synthesised with the view of mimicking hydrogen-bonding driven protein–DNA interactions and for studying the binding and cellular uptake of such polyamides (*4*–*6*). We have developed several examples of luminescent imaging and potential therapeutic agents as DNA-targeting molecules. These have been based on the use of simple heterocyclic structures (*7*), naphthalimide-derived Ru(II) complexes (*8*) and Tröger's bases (*9*), or on the use of mixed lanthanide (Yb(III) and Nd(III)) transition-metal (Ru(II)) complexes (*10*). In this study, we present the synthesis of five bis-pyridines di-peptides **1**–**5**, based on the use of acetyl-protected amino-picolinic acid, and the coupling of Boc-protected pyridine diamine to pyridine dicarboxylic acid. The solid-state structure of both **1** and **5** is also presented, which display complex crystallographic packing consisting of both intra- and intermolecular hydrogen-bonding networks. These building blocks were synthesised with the aim of investigating their ability to form a helical construct, a structural feature important for DNA interactions (see Graphical Abstract), by incorporating them into pyridine-based oligoamides (*11*). To the best of our knowledge, only a small number of pyridine diamines have been developed to date (*12*–*19*). Examples include those of Rozanski et al. (*18*) who made several pyridine oligoamides from symmetrical 2,6-diamino pyridine or 2,6-pyridine dicarboxylic acid. However, structures **1**–**5** have not to the best of our knowledge been previously formed as building blocks for the incorporation into such pyridine-based oligoamides (Figure 1).

Results and discussion

Synthesis

The synthesis of **1**–**4** required the initial preparation of acetal-protected pyridine amino acid units, possessing the carboxylic acid functionality in either the fifth or the sixth positions of the pyridine ring. For the synthesis of **1** and **2**, a two-step synthesis for *N*-acetyl-2-amino-6-picolinic acids **8** and **9** was undertaken (*17b*), Scheme 1, which initially involved the protection of 2-amino-6-picoline (**6**) and 2-amino-5-picoline (**7**) with acetic anhydride in toluene, overnight at 95°C, which yielded the two structural isomers in 87 and 90% yield, respectively (*20*). The protection of the amine was employed to prevent oxidation of this moiety and increase the stability of the resulting pyridine amino acid. Oxidation of the methyl groups gave the corresponding carboxylic acids **8** and **9** as white precipitates, in 62 and 45% yield, respectively, after reflux in water in the presence of two equivalents $KMnO_4$ and NaOH overnight, followed by acidification to pH 4 using conc. HCl. These syntheses were also repeated using

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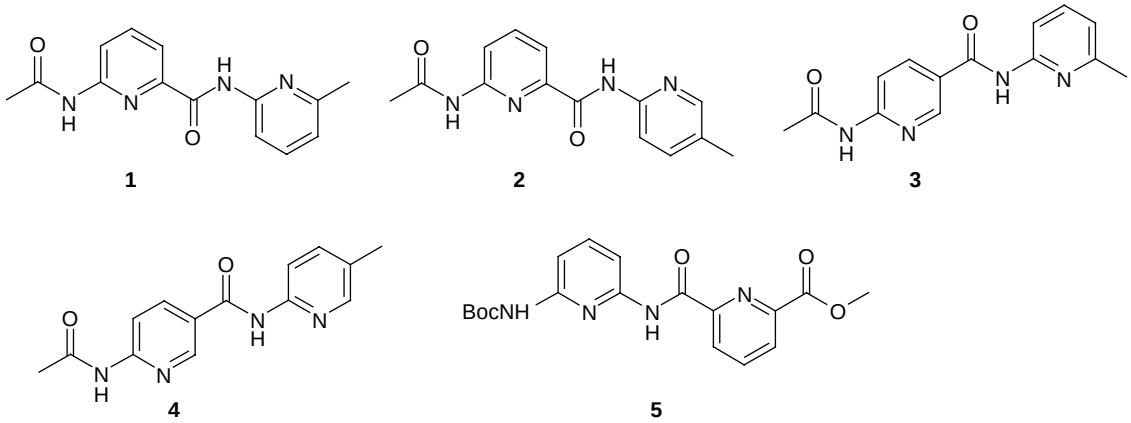
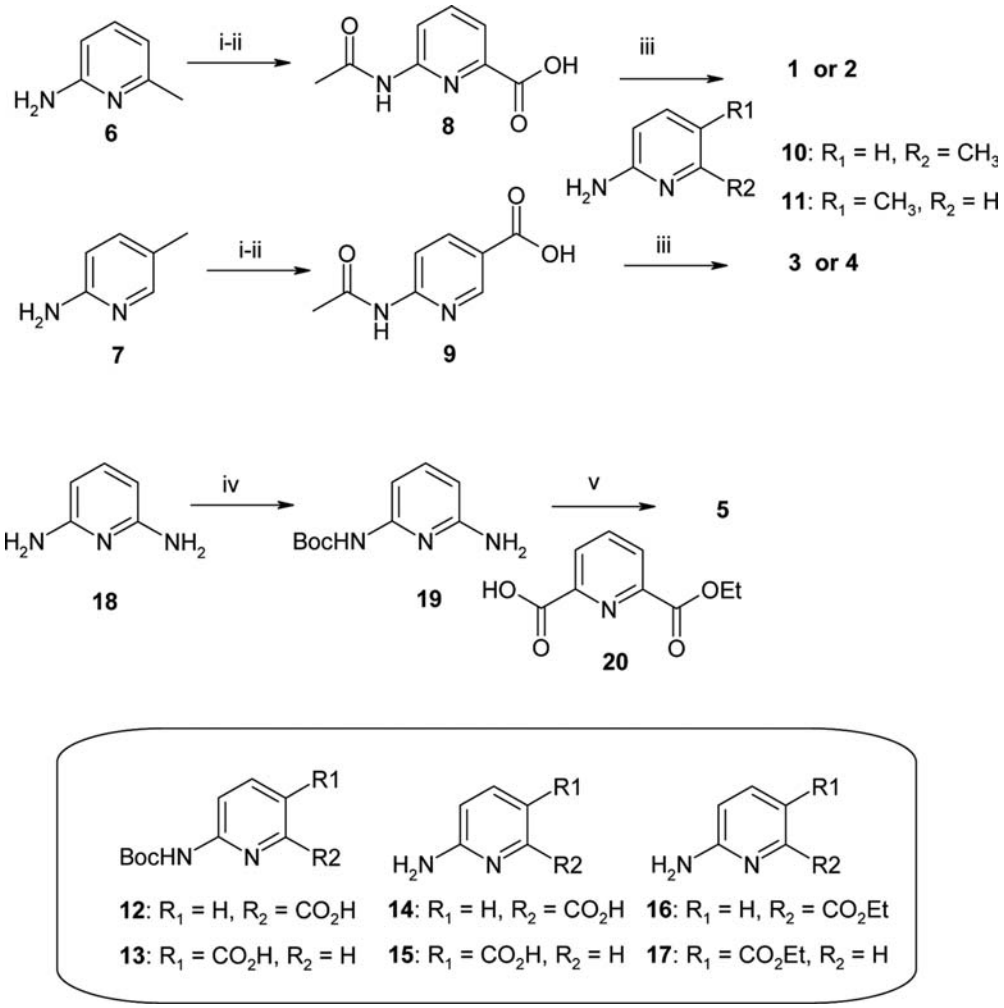


Figure 1. Structure of di-pyridine peptides **1**–**5**.



Scheme 1. (i) Acetic anhydride, toluene, 95°C. (ii) $KMnO_4$, NaOH, H_2O, reflux. (iii) DCC (or EDCI) HOBt, DMF, RT. (iv) Boc anhydride, THF, room temperature. (v) DCC, HOBt, Et_3N, THF, RT.

Table 1. Summary of crystal data, data collection and structure refinement for X-ray diffraction study of **2** and **5**.

	Compound **2**	Compound **5**
Chemical formula	$C_{14}H_{14}N_4O_2$	$C_{18}H_{20}N_4O_5$
Formula weight ($gmol^{-1}$)	270.29	372.38
Crystal system	Monoclinic	Triclinic
Space group	*P*21/*c* (no. 14)	*P*-1 (no. 2)
a (Å)	11.771(3)	7.2480(16)
b (Å)	11.903(3)	7.7467(19)
c (Å)	9.525(2)	16.949(4)
α (°)	90	82.609(6)
β (°)	92.399(5)	80.612(8)
γ (°)	90	81.488(8)
V ($Å^3$)	1333.4(6)	923.2(4)
Z	4	2
D_{calc} (g/cm^3)	1.346	1.340
μ (mm^{-1})	0.094	0.100
F (000)	568.0	392.0
Radiation (Å)	0.71073	0.71073
Temperature (K)	121(2)	121(2)
2θ Range (°)	50	50
h, *k*, *l* ranges	$-13 \rightarrow 13, -13 \rightarrow 14, -11 \rightarrow 11$	$-8 \rightarrow 8, -9 \rightarrow 9, -20 \rightarrow 20$
Measured reflections	10,397	14,143
Unique reflections	2346 ($R_{int} = 0.0910$)	3247 ($R_{int} = 0.0257$)
Reflections used [$I > 2\sigma(I)$]	1194	2927
Parameters refined	183	245
GoF (on F^2)	1.018	1.120
*R*1 [$I > 2\sigma(I)$]	0.0736	0.0408
*wR*2 [$I > 2\sigma(I)$]	0.1727	0.1201
$(\Delta\rho)_{max}/(\Delta\rho)_{min}$ ($eÅ^{-3}$)	0.315/−0.480	0.062/−0.357

Boc anhydride as the amino-protecting group, with the aim of facilitating their incorporation into oligoamides using peptide-coupling protocols. Although the two Boc-protected picolines were obtained in good yield, the oxidation of these structures to their corresponding acids **12** and **13** was unsuccessful using either $KMnO_4$ or K_2CrO_7, only resulting in the recovery of the two starting materials. With the aim of forming the free amine, which could then be coupled to **10** or **11**, the hydrolysis of **8** and **9** was also undertaken by refluxing these derivatives in 10% aqueous NaOH solution for 6 h, followed by acidic workup. This gave the corresponding pyridine amino acids **14** and **15** in 58 and 46% yield, respectively. Reaction of either **11** or **12** in a 3:20 mixture of H_2SO_4 and ethanol under reflux for 5 h under argon (*19a*), followed by aqueous NH_4^+ workup, resulted in deprotection of the *N*-acetyl group and in the esterification of the carboxylic acids, giving **16** and **17** in ca. 50% yield.

The two amino acids **14** and **15** were also reacted in excess of oxalyl chloride in $CHCl_3$ for 4 days at 45°C under anhydrous conditions, which gave their corresponding acid chlorides. Further treatment of this solution with methanol gave the corresponding methyl ester-protected pyridine amino acid analogues of **16** and **17** in ca. 50% overall yield. Having obtained several pyridine amino acid building blocks, we next formed the bis-pyridine di-peptides **1**–**4**. Initially, the acid derivatives **8** and **9** were treated with $SOCl_2$, resulting only in the recovery of the starting materials. Consequently, the peptide-coupling reagents dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) were employed. Here, **10** and **11** were coupled to **8** or **9** in DMF at room temperature using these reagents, which, after aqueous base workup and crystallisation from EtOAc, gave the desired di-peptides **1**–**4** in moderate yields. These were all characterised using conventional methods. With the view of coupling **1**–**4** to another di-peptide possessing an alternative hydrogen-donating pattern, the synthesis of the orthogonal-protected **5** was also undertaken, commencing with the mono-protection of **18**, to give **19** (*20*). As for the synthesis of bis-pyridine di-peptides **1**–**4**, **19** and mono-protected di-acid **20** (*21*) were coupled together using HOBt, DCC and triethylamine in dry THF at 0°C overnight at room temperature. The resulting residues were purified by column chromatography on flash silica (using 40/60 EtOAc/CH_2Cl_2), yielding the protected di-peptide **5** as a white solid in 27% yield.

X-ray crystallographic analysis

Crystals suitable for X-ray crystallographic analysis of **2** were grown from DMSO solution,[1] and crystallised in the monoclinic crystal system in the space group $P2_1/c$.

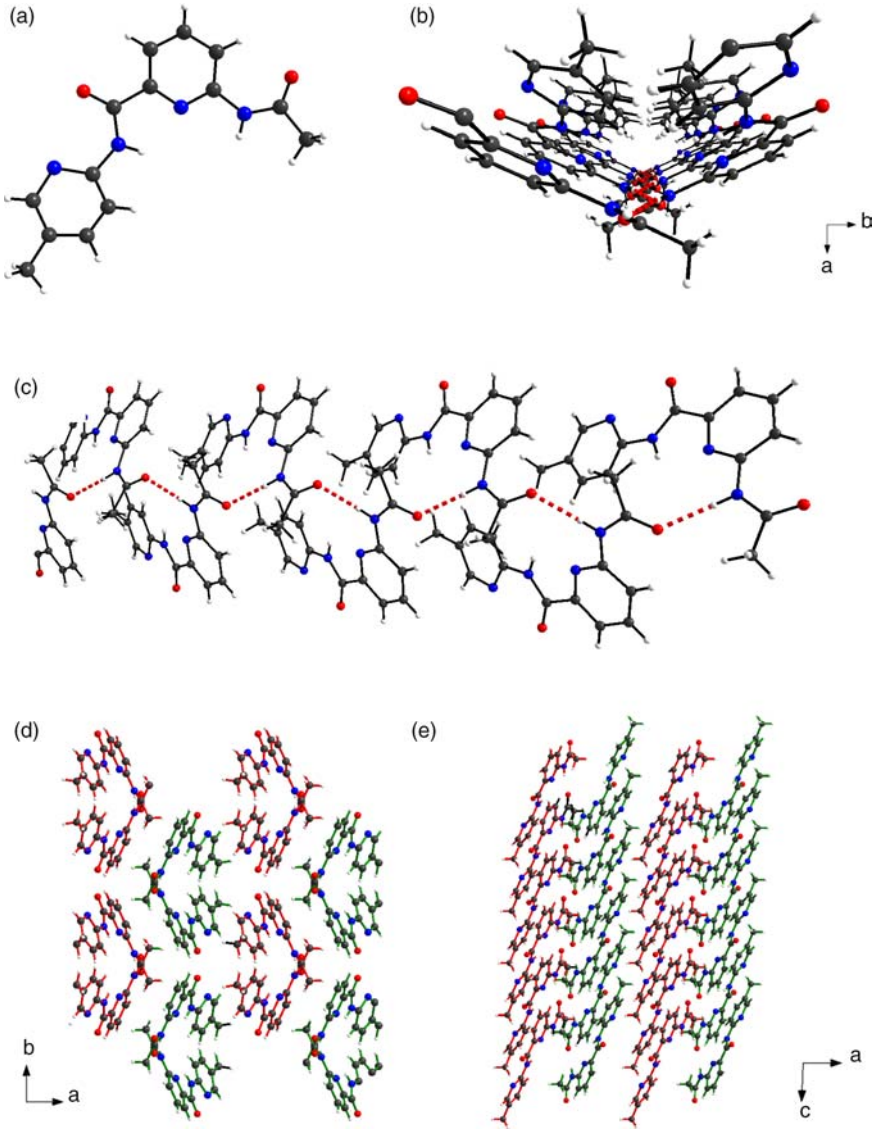


Figure 2. (a) Structure of **2**. (b) and (c) H-bonded 1D chain structure of **2**. (d) and (e) Packing diagrams with a view in the direction of the crystallographic *c*- and *b*-axes.

The crystallographic data and details of the structure refinement for **2** are provided in Table 1. The structure shows that the pyridine units are *anti* to each other in the solid state, and that the angle between the amide hydrogen atoms is 105°. On viewing the crystal structure through the 2,6-substituted pyridine plane, the second pyridine unit appears slightly twisted out of plane. The angle between the planar pyridine units is equal to the sum of three torsion angle deviations in the bonds that connect the pyridine units ($-7.3° + 4.7° + 0.2°$), which was calculated as $-2.4°$. In a similar manner, the pyrrole ring planes in the well-known DNA groove binding drug, netropsin, have been reported to have a twisted angle to one another. This angle is slightly larger than that seen herein (*23*). The difference between the pyrrole and the pyridine units shown in Figure 2, we believe, arises from the non-planarity of the pyrrole units, which is ascribed to the steric clash between the pyrrole CH and the adjacent amide group (*22*). In addition, the angle formed between the amide hydrogens was measured as 105°, suggesting that repeated units of the 2,6-substituted pyridines would span 360° after nearly five repeating units ($360°/75° = 4.8$), whereas the DNA has about 10 base pairs per turn. The results for **2** suggest that the pyridine oligoamides based on **2** could be over-curved in relation to the DNA (see Graphical Abstract). However, as mentioned earlier, both these angles and the distortion angles could change upon binding in the minor groove, as has been shown to be the case for netropsin (*23*).

The supramolecular arrangement of **2** and the involved intermolecular forces might give further indications of

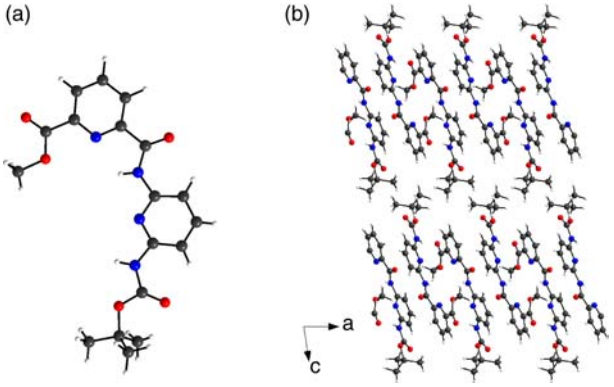


Figure 3. (a) Structure of **5**. (b) Packing of **5** in the crystal structure with a view in the direction of the crystallographic *b*-axis (C: grey, O: red, N: blue and H: white) (colour online).

how this compound could interact with DNA, and these were investigated by looking at the packing arrangement of the molecule. The supramolecular structure of **2** is characterised by intermolecular H bonds involving the N and O atoms of the amide functionality that bridges the aromatic moieties. This hydrogen bond results in a 1D chain structure that runs in the direction of the crystallographic *c*-axis. The distance between the hydrogen donor N atom and the O acceptor atom is 2.935(5) Å. The alkyl moiety of the terminating ester functionality and the methyl substituent of the aromatic ring prohibit strong $\pi-\pi$ interactions in the crystal structure. Figure 2(b) and (c) shows the 1D H-bonded chain structure; Figure 2(d) and (e) highlights the arrangement of the chain within the crystal structure.

Crystals suitable for X-ray crystal structure analysis of **5** were obtained by slow evaporation from a methanol solution. Compound **5** crystallises in the triclinic crystal system in the space group *P*-1. The resulting structure is shown in Figure 3(a). The crystallographic data for **2** are provided in Table 1. The crystal structure consists of isolated molecules, held together through weak dispersion forces. In contrast to the crystal structure of **2**, shown in Figure 2(a), the crystal structure of **5** shows the pyridine rings *syn* to each other. This orientation and binding pattern (H-donor/H-acceptor) could preferentially target (i) **G** sequences, by hydrogen bonds between the pyridine nitrogen and the NH_2 of **G** and by (ii) the amide hydrogen and the lone pair of the **G** base (see Graphical Abstract). As for the crystal structure of **2**, the angle between the relatively planar pyridine aromatic rings was calculated from the three torsion angles that connect the pyridine units ($-1.28°$, $-0.56°$, $+1.48°$) as $-0.36°$, which is of similar magnitude to that seen for **2**, demonstrating a small rise between the two pyridine units. Furthermore, the angle formed between the two 'amide' protons was determined as 130°, which is somewhat larger than that seen for **2**. These results indicate that the configuration of the 2,6-diaminopyridine units would span

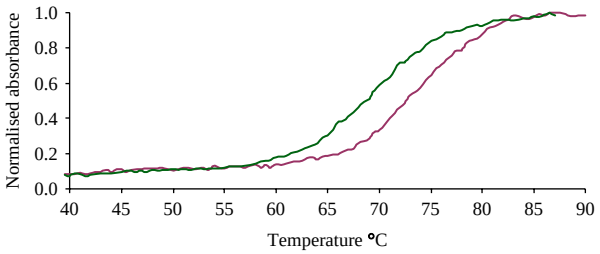


Figure 4. Thermal denaturation curves of *ct*-DNA (150 μM) in 10-mM phosphate buffer, pH 7.4, in the absence (green) and presence of **2** (purple) at a P/D of 10 (colour online).

360° after seven units (360°/50° = 7.2), an improvement on that seen for **2**. The supramolecular arrangement of **5** was also analysed. Figure 3(b) shows the packing of **5** with a view in the direction of the crystallographic *b*-axis. Unlike that seen for **2** mentioned above, the intramolecular arrangement of H-donor and acceptor atoms within **5** does not allow the molecule to be involved in extended H-bonded networks. Furthermore, the alkyl substituents of the ester functionalities hamper efficient $\pi-\pi$ stacking in the crystal structure.

Biological studies

With the aim of evaluating the potential binding of **2** to DNA, we carried out thermal denaturation experiments. A typical melting curve for *ct*-DNA in aqueous buffered solvent at a physiological pH of 7.4 is shown in Figure 4, from which the melting temperature T_m for calf thymus *ct*-DNA was determined as 69°C. When a small molecule binds to DNA, it either stabilises or destabilises the double helix and thus modulates the T_m number. Denaturation studies were conducted in 10-mM phosphate buffer (pH 7.4) with a P/D ratio of 10. As shown in Figure 4, it is clear that the binding of **2** stabilises the DNA structure, with a T_m value of 75°C determined (from the first derivative plots of the melting curve) for the binding of **2**, which is of similar magnitude seen for the binding of many positively charged organic molecules or transition-metal complexes to *ct*-DNA (*8*).

To evaluate if these structures could bind to DNA *in situ* and/or induce apoptosis in cancer cell lines, the cytotoxicity of the di-peptides **1**–**4** was investigated against drug-resistant chronic myeloid leukaemia K562 cell-line. These acetate-protected di-peptides were only found to be moderately soluble in water. However, the incubation of **2** with K562 for 48 h and the subsequent assessment of efficacy by the MTT assay demonstrated that **2** was indeed cytotoxic. Here, compound **2** was shown to reduce cell numbers at relatively high doses, with an EC_{50} value of 82 μM, and cell morphology indicated that the cells had undergone apoptosis. Hence, this result indicates that these structures are able to cross the cell

membrane and induce cellular death at high concentrations. This makes them excellent candidates for incorporation into oligoamides with the view of achieving site-selective targeting of DNA *in situ.*

Conclusion

In summary, we have undertaken the synthesis of several pyridine amino acid derivatives and bis-pyridine di-peptides for the formation or incorporation into pyridine-based oligoamides. We have shown, using X-ray crystal structure analysis, that the bis-pyridines di-peptides **2** and **5** have curved molecular structures, which could facilitate the formation of helically shaped pyridyl oligoamides. The supramolecular structure of **2** is characterised by 1D chains which runs parallel to the crystallographic *c*-axis and in which single molecules are connected through H-bonds. In contrast to **2**, the arrangement of N-atoms in **5** does not allow the molecule to be engaged in strong intermolecular H-bonds. The packing of **5** in the crystal structure is characterised by isolated molecules held together through weak dispersion forces. We have further shown that in the case of **2**, the binding of the oligopyridine units stabilises the DNA structure at the same time, as we demonstrated that **2** entered the cells and gave rise to apoptosis with an EC_{50} value of 82 μM in a drug-resistant chronic myeloid leukaemia K562 cell-line. We are currently undertaking the synthesis of other oligoamide derivatives based on these building blocks presented herein and investigating their binding to DNA as well as their cytotoxicity in cancer cell lines.

Experimental

General

Reagents (obtained from Aldrich) and solvents were purified using standard techniques. Melting points were determined using a GallenKamp melting point apparatus. Infrared spectra were recorded on a Mattson Genesis II FTIR spectrophotometer equipped with a Gateway 2000 4DX2-66 workstation. ^{1}H NMR spectra were recorded at 400 MHz using a Bruker Spectrospin DPX-400 instrument. ^{13}C NMR spectra were recorded at 100 MHz using a Bruker Spectrospin DPX-400 instrument.

Synthesis

*6-Acetylamino-pyridine-2-carboxylic acid (6-methyl-pyridin-2-yl)-amide (**1**)*

Compound **8** (0.28 g, 1.57 mmol) along with 2-amino-6-picoline (0.21 g, 1.88 mmol) and HOBt (0.25 g, 1.84 mmol) was dissolved in DMF. To this solution, DCC (0.35 g, 1.67 mmol) was added at 0°C. The reaction mixture was stirred overnight under argon at room temperature. The reaction mixture was then kept at 4°C for 3 h before filtering it through celite. The solvent was evaporated under reduced pressure and the product was obtained as a dried white solid (0.06 g, 0.22 mmol, 14%) that was dried under high vacuum. M.p. 205–207°C; ^{1}H NMR δ_H (400 MHz, DMSO-d_6): 10.9 (1H, s, Ar—NH—CO—Ar), 10.2 (1H, s, Ar—NH—CO—CH_3), 8.34 (1H, d, $J = 8.4$ Hz, Ar—H), 8.11 (1H, d, $J = 8.0$ Hz, Ar—H), 8.03 (1H, t, $J = 8.0$ Hz, Ar—H), 7.86 (1H, d, $J = 7.6$ Hz, Ar—H), 7.77 (1H, t, $J = 7.8$ Hz, Ar—H), 7.05 (1H, d, $J = 7.2$ Hz; Ar—H), 2.43 (3H, s, CO—CH_3), 2.16 (3H, s, Ar—CH_3); ^{13}C NMR δ_C (100 MHz, DMSO-d_6), 169.6, 161.6, 157.0, 151.0, 150, 146.8, 140.4, 139.0, 119.5, 117.0, 116.9, 110.0, 24.0, 23.6; accurate MS (*m*/*z*) calculated: 271.1190 $(M + H)^+$, found: 271.1195; IR ν_{max} (cm^{-1}): 3354 (NH), 3251 (NH), 1689 (C═O), 1668 (C═O), 1449 (pyridine).

*6-Acetamido-N-(5-methylpyridin-2-yl)picolinamide (**2**)*

Compound **8** (0.61 g, 3.4 mmol), 2-amino-5-picoline (0.44 g, 4.1 mmol) and HOBt (0.15 g, 4.0 mmol) were dissolved in DMF. DCC (0.76 g, 3.7 mmol) was dissolved in CH_2Cl_2 and added at 0°C under argon. The reaction mixture was stirred for 2 h and then additional DCC (0.38 g, 1.8 mmol) was added, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was then filtered through celite and the filtrate was evaporated under reduced pressure. After purification by silica flash column chromatography eluting with 20% EtOAc/CH_2Cl_2, the product was obtained as a white solid (0.15 g, 0.56 mmol, 16%). M.p. 204–206°C; ^{1}H NMR δ_H (400 MHz, DMSO-d_6): 10.9 (1H, s, NH—CO—Pyr), 10.4 (1H, s, CO—NH—Me), 8.34 (1H, d, $J = 8.0$ Hz, Ar—H), 8.24 (1H, s, Ar—H), 8.20 (1H, d, $J = 8.4$ Hz, Ar—H), 8.06 (1H, t, $J = 8.0$ Hz, Ar—H), 7.87 (1H, d, $J = 7.6$ Hz, Ar—H), 7.73 (1H, dd, $J = 8.4$; 2.0 Hz, Ar—H), 2.30 (3H, s, Ar—CH_3), 2.16 (3H, s, CH_3—CO); ^{13}C NMR δ_C (100 MHz, DMSO-d_6): 169.6, 161.4, 156.6, 151.0, 148.2, 146.8, 140.5, 139.0, 119.5, 117.0, 116.9, 110.0, 24.0, 23.6; IR ν_{max} (cm^{-1}): 3354 (NH), 3252 (NH), 1689 (C═O), 1668 (C═O).

*6-Acetamido-N-(5-methylpyridin-2-yl)nicolinamide (**3**)*

Stirred solution of **9** (0.18 g, 1.0 mmol), 2-amino nicoline (0.12 g, 1.1 mmol) and HOBt (0.17 g, 1.3 mmol) was suspended in DMF, and DCC (0.26 g, 1.3 mmol) and triethylamine were added and stirred for 30 min at 0–4°C under argon and then at room temperature overnight. The reaction mixture was filtered through celite and the solvent was evaporated under reduced pressure. After purification by silica flash column chromatography eluting with 20%

EtOAc/CH_2Cl_2 → 50% EtOAc/CH_2Cl_2, the product was obtained as a white solid (0.06 g, 0.24 mmol, 24% yield). M.p. 217–218°C; ^{1}H NMR δ_H (400 MHz, DMSO-d_6): 10.9 (1H, s, Ar—NH—CO—Pyr), 10.2 (1H, s, Ar—NH—CO—Me), 8.34 (1H, d, $J = 8.4$ Hz, Ar—H), 8.11 (1H, d, $J = 8.0$ Hz, Ar—H), 8.03 (1H, t, $J = 8.0$ Ar—H), 7.86 (1H, d, $J = 7.6$ Hz, Ar—H), 7.77 (1H, t, $J = 8.0$ Hz, Ar—H), 7.05 (1H, d, $J = 7.2$ Hz, Ar—H), 2.43 (3 H, s, Ar—CH_3), 2.16 (3 H, s, CH_3—CO); ^{13}C NMR δ_C (100 MHz, DMSO-d_6): 170.0, 161.6, 157.0, 151.0, 150.0, 146.8, 140.3, 139.0, 119.5, 117.2, 116.9, 110.0, 24.0, 23.6; accurate MS (*m/z*) calculated: 293.1008 $(M + Na)^+$, found: 293.1014; IR ν_{max} (cm^{-1}): 3355 (NH), 3251 (NH), 1689 (C═O), 1668 (C═O), 1447 (pyridine).

*6-Acetamido-N-(6-methylpyridin-2-yl)nicotinamide (**4**)*

Compound **9** (0.18 g, 1.0 mmol), 2-amino-5-picoline (0.15 g, 1.0 mmol) and HOBt (0.15 g, 1.1 mmol) were weighed out and dried under reduced pressure before being dissolved in dry THF (10 ml) and cooled in an ice bath for 30 min. EDCI HCl (0.21 g, 1.1 mmol) was added and stirred for further 30 min at 0°C and then stirred overnight at room temperature. The solvent was removed and the solid was dried under reduced pressure before CH_2Cl_2 was added. The mixture was washed three times with saturated $NaHCO_3$ and the organic layer was dried over $MgSO_4$, filtered and evaporated to give white crystals (0.04 g, 0.16 mmol, 16% yield). M.p. 216–217°C; ^{1}H NMR δ_H (400 MHz, DMSO-d_6): 10.84 (1H, s, CO—NH—Pyr), 10.83 (1H, s, CO—NH—Me), 8.93 (1H, d, $J = 2.4$ Hz, Ar—H), 8.37 (1H, dd, $J = 8.8$; 2.8 Hz, Ar—H), 8.22 (1H, s, Ar—H), 8.16 (1H, d, $J = 8.8$ Hz, Ar—H), 8.08 (1H, d, $J = 8.4$ Hz, Ar—H), 7.67 (1H, dd, $J = 8.4$; 2.0 Hz, Ar—H), 2.29 (3H, s, Ar—CH_3), 2.14 (3H, s, CH_3—CO); ^{13}C NMR δ_C (100 MHz, DMSO-d_6): δ 169.8, 163.9, 154.2, 149.9, 148.3, 147.7, 138.5, 138.1, 128.9, 125.0, 114.3, 112.0, 24.1, 17.4; accurate MS (*m/z*) calculated: 293.1008 $(M + Na)^+$, found: 293.1014; IR ν_{max} (cm^{-1}): 3355 (NH), 3251 (NH), 1689 (C═O), 1668 (C═O), 1449 (pyridine).

*Methyl 6-(6-(tert-butoxycarbonylamino)pyridin-2-ylcarbamoyl)-picolinate (**5**)*

Stirred solution of **19** (0.50 g, 2.4 mmol), **20** (0.50 g, 2.8 mmol) and HOBt (0.38 g, 2.8 mmol) was suspended in THF. Triethylamine (0.5 ml, 2.6 mmol) and DCC (0.55 g, 2.4 mmol) were added to this solution at 4°C and under argon. This reaction mixture was stirred at 4°C for 15 min and then overnight at room temperature. The reaction mixture was filtered and the solvent was removed under reduced pressure. The reaction mixture was dried and then suspended in CH_2Cl_2. The residue was separated by silica flash column chromatography (4% EtOAc/CH_2Cl_2), resulting in the product obtained as a white solid (0.24 g, 0.64 mmol, 27%). M.p. 153.2–154.0°C; ^{1}H NMR δ_H (400 MHz, DMSO-d_6): 10.1 (1H, s, NH), 9.98 (1H, s, NH), 8.42 (1H, dd, $J = 6.8$; 2.4 Hz, Ar—H), 8.33–8.30 (2H, m, 2 × Ar—H), 7.92 (1H, dd, $J = 8.0$; 2.0 Hz, Ar—H), 7.83 (1H, t, $J = 7.8$ Hz, Ar—H), 7.63 (1H, dd, $J = 8.0$; 0.8 Hz, Ar—H), 3.37 (3H, s, CH_3), 1.48 (9H, s, $(CH_3)_3$); ^{13}C NMR δ_C (100 MHz, DMSO-d_6): 178.3, 164.6, 153.1, 152.0, 149.2, 141.1, 128.7, 126.0, 109.0, 107.4, 80.1, 68.8, 53.4, 28.5, 27.9, 22.2; IR ν_{max} (cm^{-1}): 3344 (NH_2), 1693 (C═O).

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Note

1. The data for crystal **2** (CDC 743207) was collected on a Rigaku Saturn 724 CCD Diffractometer. A suitable crystal was mounted using inert oil on a 0.30 mm quartz fibre tip and immediately placed on the goniometer head in a 121K N_2 gas stream. The data set was collected using Crystalclear-SM 1.4.0 software and 1926 diffraction images, of 0.5° per image, were recorded. Data integration and reduction were performed using Crystalclear-SM 1.4.0 software. The data for crystal **5** (CCDC 743208) was collected on a Bruker Smart Apex Diffractometer. A suitable crystal was mounted using inert oil on a glass fibre tip and immediately placed on the goniometer head in a 121K N_2 gas stream. The data was collected using Bruker Smart Version 5.625 software run in multirun mode and 2400 image frames, of 0.3° per frame, were recorded. Data integration and reduction were carried out using Bruker Saint + Version 6.45 software and corrected for absorption and polarisation effects using Sadabs Version 2.10 software.

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